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Identification of *Burkholderia* and *Penicillium* isolates from kauri (*Agathis australis*) soils that inhibit the mycelial growth of *Phytophthora agathidicida*

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Abstract *Phytophthora agathidicida* is a highly virulent pathogen of kauri (*Agathis australis*) and the causal agent of dieback disease in New Zealand's kauri forests. This study aimed to identify microbial isolates isolated from kauri forest soils that inhibited the growth of *P. agathidicida.* Three different forms of *in vitro* bioassays were used to assess the inhibition of each isolate on the mycelial growth of *P. agathidicida*. Furthermore, head space (HS) solid-phase micro-extraction coupled with gas chromatography-mass spectrometry (SPME-GC-MS) was performed to identify if the microbial isolates emitted volatile organic compounds (VOCs), which may be contributing to inhibition. This research identified several bacterial isolates belonging to the genus *Burkholderia* that inhibited the mycelial growth of *P. agathidicida*. Furthermore, several VOCs produced by these isolates were putatively identified, which may be responsible for the inhibition observed in the bioassays. Several isolates of *Penicillium* were identified that inhibit *Phytophthora agathidicida*, with the culture filtrate of one isolate being found to strongly inhibit *P. agathidicida* mycelial growth. These isolates of *Burkholderia* and *Penicillium* appear to exhibit multiple modes of antagonism against *P. agathidicida*, including microbial competition and the production of diffusible and volatile anti-microbial compounds. Although further research is needed to better define their mechanisms of inhibition, these findings have identified candidate microbial antagonists of *P. agathidicida*.

Keywords *Phytophthora agathidicida*; *Burkholderia*; *Penicillium*; headspace solid-phase micro-extraction gas chromatography-mass spectrometry (HS SPME-GC-MS) analysis; volatile organic compounds; *in vitro* bioassay

INTRODUCTION

The spread of kauri dieback, caused by the pathogen *Phytophthora agathidicida*, currently threatens the long-term health of New Zealand's kauri (*Agathis australis*) forests (Beever et al. 2009). Kauri dieback was first recognised to be afflicting kauri on Great Barrier Island (Hauraki Gulf, Auckland) in the 1970s, and is now widespread across most mainland kauri forests (Gadgil 1974; Waipara et al. 2013). Kauri dieback is a root and collar rot disease that is spread through the movement of soil and root pieces infested with *P. agathidicida* (Bellgard et al. 2016). Current management strategies primarily focus on disease containment by preventing further spread of *P. agathidicida* infested soils into new forested areas (Bradshaw et al. 2020). Several research efforts have been made to manage the disease which include phosphite injections directly into trees (Horner & Hough 2013), oospore deactivation (Dick & Kimberley 2013), screening kauri for genetic resistance (Herewini et al. 2018) and mātauranga Māori guided discovery of native plant bio-actives with anti-*Phytophthora* properties (Lawrence et al. 2019).

Despite these scientific studies, there has been limited research on how the soil microbiota may suppress kauri dieback by antagonising *P. agathidicida*. Several plant diseases caused by *Phytophthora* pathogens have, in part, been managed using chemical control, such as potato (*Solanum tuberosum*) late blight caused by *P. infestans* (Majeed et al. 2017) and pepper (*Capsicum annuum*) blight caused by *P. capsici* (Kim et al. 2010). However, there are concerns over the widespread use of chemical control due to the associated environmental impacts and the development of fungicide resistance (O'Brien 2017). Disease management strategies that integrate the use of microbial agents to antagonise pathogens and prevent plant infection are considered to be a more sustainable, less environmentally impactful alternative (Bhusal & Mmbaga 2020). For the management of soil-borne plant pathogens, it is preferential to target the discovery of microbial antagonists to the host

associated soil microbiota. Members of the resident soil microbiota are more likely to establish, proliferate and elicit the functional traits required to suppress plant disease when applied in field settings (Raaijmakers et al. 2009; Zohara et al. 2016).

Previous research by Byers et al. (2020) identified significant differences in the composition of fungal and bacterial communities associated with asymptomatic and symptomatic kauri trees across Waipoua Forest (Northland Region, New Zealand). Several of the microbial taxa found in significantly higher relative abundance in asymptomatic kauri soils have been previously reported to inhibit various other *Phytophthora* pathogens. These include genera such as *Penicillium* (Fang & Tsao, 1995; Ma et al., 2008), *Trichoderma* (Ahmed et al. 2000; Widmer 2014; Bae et al. 2016), and *Pseudomonas* (Tran et al. 2007; Zohara et al. 2016; Caulier et al. 2018). Although Byers et al. (2020) identified microbial taxa associated with potentially disease suppressive soils (i.e. asymptomatic host states), it did not assess whether these taxa exhibit an antagonistic interaction against *Phytophthora agathidicida*. Therefore, additional research is needed to isolate and identify members of the kauri soil microbiota that can be demonstrated to inhibit the growth of *P. agathidicida*[1].

In this study, we aimed to isolate microorganisms from soils associated with asymptomatic kauri to identify if any suppressed the mycelial growth of *P. agathidicida*. Three different bioassay types were used to assess each microbial isolate: dual culture bioassays, culture filtrate bioassays, and split plate bioassays. Dual culture bioassays were selected as the primary form of bioassay because they were a fast and easily reproducible method to screen many microbial isolates against *P. agathidicida* (Kunova et al. 2016; Zohara et al. 2016). To provide more insight into the mechanisms driving mycelial inhibition, culture filtrate bioassays were used to assess whether diffusible compounds produced by the microbial isolates were contributing to inhibition (Ma et al. 2008; Zohara et al. 2016). In addition, split plate bioassays were used as a method to assess whether the microbial isolates were producing volatile organic compounds (VOCs) that may be inhibiting *P. agathidicida* (Syed-Ab-Rahman et al. 2019).

Volatile organic compounds (VOCs) are secondary metabolites released by soil microorganisms that are known to exhibit anti-microbial properties against a wide range of plant pathogens (de Boer et al. 2019). Due to their low molecular mass, high vapour pressure and low boiling point (Insam & Seewald 2010; Piechulla & Degenhardt 2014), microbial VOCs can readily evaporate and diffuse through the soil to interact with other soil microorganisms (Effmert et al. 2012; Bitas et al. 2013; Schmidt et al. 2015). VOCs have been demonstrated to inhibit several *Phytophthora* pathogens including *P. infestans* (Hunziker et al. 2015; Kumar et al. 2018; Elsherbiny et al. 2020), *P. capsici* (Syed-Ab-Rahman et al. 2019), and *P. cinnamomi* (Méndez-Bravo et al. 2018). This study used solid-phase micro-extraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS) to extract and identify VOCs from the headspace (HS) of the isolates found to inhibit *P. agathidicida*, so that the potential mechanisms driving inhibition could begin to be explored.

[1] The genus name *Phytophthora* has been abbreviated to *P.* in instances where it would not be confused with another genus. All other genera starting with the letter P are spelt out in full throughout the text.

MATERIALS AND METHODS

Isolation of microorganisms from kauri soil

The microbial isolates screened against *P. agathidicida* during this study were isolated from organic layer soils collected from asymptomatic kauri trees across Waipoua Forest (Northland Region, New Zealand). Soil samples were collected during previous soil sampling as outlined by Byers et al. (2020). Following sampling, soils were temporarily stored at 4°C in darkness prior to use in this study. One of the wider aims of this kauri dieback project was to understand the microbiome associated with soils associated with asymptomatic kauri trees, i.e. those near diseased trees in infected kauri stands (Byers et al 2020). Therefore, only kauri soil samples that tested negative for the presence of *P. agathidicida* when screened using a soil baiting bioassay and real-time PCR assay (Than et al. 2013; McDougal et al. 2014) were used in this study.

Microbial isolates were isolated from kauri soils using soil serial dilutions and selective agar plating (Zohara et al. 2016). Eight soil samples were randomly selected from the store of uninfected kauri soil outlined above. Per sample, triplicate 1 g subsamples were suspended in 100 mL of autoclaved double-distilled (dd) H_2O and shaken at 140 rpm on an orbital shaker (Ratek Instruments Pty Ltd., Australia) for 4 hours at room temperature before being left to settle for 1 hour. Once settled, 1 mL of each soil suspension was diluted at a 1 : 10 concentration with autoclaved ddH_2O. Following this, 1 mL of each serial dilution was plated onto a bacterial selective and a fungal selective agar plate and spread evenly across the surface of the plate using a cell spreader. Recipes for the bacterial selective and fungal selective agar plates are listed in the Supplementary Methods.

Selective agar plates were incubated for up to 4 days in darkness at 21.5°C, which is the optimum growth temperature of *P. agathidicida* (Weir et al. 2015). This incubation temperature was selected to cultivate microbial isolates compatible for growth in bioassay screenings with *P. agathidicida*. Plates were inspected every 24 hours, with emerging fungal and bacterial colonies immediately sub-cultured onto potato dextrose agar (PDA) (Oxoid Ltd., UK) and nutrient agar (NA) (Oxoid Ltd., UK), respectively. By the end of this selective isolation process, 164 bacterial isolates and 170 fungal isolates were isolated from and used for pre-screening assays. Microbial isolates were temporarily stored on half strength PDA or NA plates in darkness at 4°C before being screened.

P. agathidicida source material

The *P. agathidicida* strain NZFS3770 was used to conduct experiments throughout the duration of this study. This isolate was cultured on 10% carrot agar, ampicillin, nystatin, rifampicin, pimaricin and hymexazol (CRNH) selective medium (Herewini et al. 2018) to preserve pure cultures and maintained on 20% clarified V8 agar in darkness at 21.5°C (Lawrence et al. 2017).

Pre-screening of microbial isolates

Due to the high number of isolates that required screening, pre-screening rounds were conducted before beginning more detailed bioassays. For pre-screenings, dual culture bioassays were prepared in triplicate for each microbial isolate. These dual culture bioassays are fully described in the following Section. Only isolates that reduced the mycelial growth (mm) of *P. agathidicida* when compared to the control were retained for further bioassay analysis using more dual culture bioassays, culture filtrate bioassays and split plate bioassays.

Dual culture bioassays

Microbial isolates that passed pre-screening were screened against *P. agathidicida* again using dual culture bioassays at a higher replication number. To prepare bacterial isolates for the dual culture bioassays, each isolate was streaked out onto an NA plate and incubated for in darkness at 21.5°C for 24 hours. Single colonies were inoculated into 15 mL of sterile tryptone soya broth (TSB) (Oxoid Ltd., UK) using a 1-µL inoculation loop. Broth cultures were shaken at 100 rpm on an orbital shaker (Ratek Instruments Pty Ltd., Australia) in darkness at 21.5°C for 48 hours. After incubation, broth cultures were centrifuged (4000 rpm for 20 minutes) and the supernatant was discarded. Bacterial pellets were suspended in 500 µL of autoclaved ddH_2O. To prepare the fungal isolates and *P. agathidicida* for dual culture bioassays, a 5-mm diameter agar plug of each isolate was sub-cultured onto a fresh PDA plate and incubated in darkness at 21.5°C for 5 days.

To set up the dual culture bioassays, a 5-mm diameter agar plug of *P. agathidicida* was taken from the leading mycelial edge and placed into the centre of a PDA plate. For each fungal isolate, two 2.5-mm diameter agar plugs were positioned 2 cm away from the *P. agathidicida* agar plug and on opposite sides of the PDA plate. For bacterial isolates, PDA plates were inoculated with two 50 µL doses of liquid culture in the same positions as described for fungal isolates. Control plates were prepared by inoculating PDA plates that contained a 5-mm diameter agar plug of *P. agathidicida* with either two 2.5-mm^2 blank PDA agar plugs or two 50-µL doses of autoclaved ddH_2O. A graphical description of this experimental set up can be seen in Fig. S1 (Supplementary Materials).

Culture filtrate bioassays

Broth cultures of each bacterial isolate were prepared as described in the previous section. Following incubation at 21.5°C for 48 hours, bacterial cultures were centrifuged (4000 rpm for 40 minutes) and the resulting supernatant was double filtered using cellulose acetate ReliaPrep™ 0.2 µM syringe filters (Ahlstrom-Munksjö, Finland) to a obtain a cell-free filtrate. Methods for the fungal isolates followed those described above for bacterial isolates. However, fungal broth cultures were prepared by inoculating 25 mL of sterile malt extract broth (MEB) (Oxoid Ltd., UK) with three 5-mm diameter agar plugs of each fungal isolate which were then incubated in darkness at 25°C for 5 days.

To set up the culture filtrate bioassays, one 5-mm diameter agar plug of *P. agathidicida* was sub-cultured onto a PDA plate. Following this, 500 µL of either bacterial or fungal cell free filtrate was pipetted directly onto the *P. agathidicida* agar plug (Vinale et al. 2006; Ma et al. 2008; Zohara et al. 2016). Control plates consisted of one 5-mm diameter agar plug of *P. agathidicida* which was inoculated with either 500 µL of sterile TSB or MEB.

Split plate bioassays

Methods for split-plate bioassays were modified based methods outlined by Syed-Ab-Rahman et al. (2019). Split-plate Petri dishes (Thermo Fisher Scientific, New Zealand) which had a 0.5-mm wide strip of agar cut out to further separate each microbial isolate from *P. agathidicida* were used to set up the bioassays. Bacterial broth cultures and fungal agar cultures were prepared as described in the section on dual culture bioassays. Following preparation, either 100 µL of bacteria or a 5-mm diameter agar plug of fungi was inoculated onto one side of a split PDA plate. The opposite side of the split PDA plate was then inoculated with a 5-mm diameter agar plug of *P. agathidicida*. Example images of bacterial split plate bioassays can be seen in Fig. S2 (Supplementary Materials).

Calculation of *P. agathidicida* inhibition

Following preparation, all bioassays were incubated in darkness at 21.5°C for 7 days before *P. agathidicida* was measured for its mycelial growth (mm). Each of the three different bioassay types were repeated for three rounds and for each of the three rounds, five replicates were performed per microbial isolate. Following incubation, the mycelial inhibition (MI) value (%) of each bioassay was calculated using the formula: *'((C - A) / C) x 100)'*. Where, C is the growth (mm) of *P. agathidicida* on control plates and A is the growth (mm) of *P. agathidicida* on plates inoculated with a microbial isolate. Significant differences (p-value < 0.05) in MI values (%) between experimental and control bioassays were determined using Students T-tests (p-value < 0.05).

Profiling of VOCs using HS SPME-GC-MS

Headspace solid-phase micro-extraction coupled with gas chromatography-mass spectrometry (HS SPME-GC-MS) was performed to profile the VOCs released by the microbial isolates. To prepare for analysis, fungal isolates were sub-cultured onto a PDA plate and incubated at 21.5°C in darkness for 7 days. Following this, four 5-mm diameter agar plugs were transferred into a 20 mL amber glass headspace vial (Supelco Analytical, Sigma Aldrich) and incubated at 21.5°C for 24 hours. Liquid cultures of bacterial isolates were prepared as described in the section on dual culture bioassays. Following preparation, 100 µL of bacteria was inoculated into a headspace vial containing 3 mL of NA and incubated at 21.5°C for 24 hours. To prepare *P. agathidicida* only control vials, an agar plug of *P. agathidicida* was freshly sub-cultured onto PDA and incubated in darkness at 21.5°C for 5 days. Following incubation, four 5-mm diameter agar plugs were transferred into a headspace vial and incubated at 21.5°C for 24 hours. As further controls, headspace vials containing either four 5-mm diameter blank PDA plugs or 3 mL of NA inoculated with autoclaved ddH_2O were prepared and incubated at the same conditions

as the sample vials. Three replicate headspace vials were set up for all experimental and control samples analysed. During incubation, headspace vials were plugged with a sterile cotton ball to maintain aerobic conditions. Following incubation, cotton ball plugs were discarded, and headspace vials were sealed with an 18-mm thread magnetic screw cap that contained a 1.5 mm thick blue PTFE/silicone septum (Supelco Analytical, Sigma Aldrich).

Methods for the HS SPME-GC-MS analysis followed those outlined by Stoppacher et al. (2010) and Nieto-Jacobo et al. (2017). A Shimadzu QP2010 gas chromatograph-mass spectrometer (Shimadzu Corporation, Japan) fitted with a CTC-CombiPAL XYZ auto sampler and a Restek Rxi-5 ms fused silica capillary column (HP5-MS 30 m × 0.25 mm × 0.25 µm, Bellefonte, PA, USA) was used to perform the analysis. VOCs were extracted from the sample headspace vials for 30 minutes without agitation using an SPME fibre that had a 65 µM polydimethylsiloxane/divinylbenzene (PDMS/DVB) coating. After injection, volatiles bound to the fibre were desorbed for 2 minutes in a split/split less injector at 250°C. The oven temperature programme was held at 40°C for 2 minutes; raised 10°C per minute to 200°C; further raised 25°C per minute to 260°C and then held at 260°C for 5 minutes. Helium was used as carrier gas at a constant flow rate of 1 mL per minute.

The acquisition and processing software GCMS solution version 4.45 (Shimadzu Corporation, Japan) was used to putatively identify the best match for each detected compound. This software used the NIST 2011 and Wiley 10 mass spectral libraries to identify compounds in conjunction with their reported linear retention index (LRI) information. An alkane standard solution C_8-C_{20} (Sigma-Aldrich, Australia) was run to calculate LRI values for each identified compound. The reported LRI values for each compound were compared to previously reported literature LRI values, which were obtained from ChemSpider (http://www.chemspider.com/), NIST Chemistry WebBook (https://webbook.nist.gov/chemistry/), PubChem (https://pubchem.ncbi.nlm.nih.gov/), and Pherobase (https://www.pherobase.com/) (Table S1, Supplementary Materials).

Only compounds with an LRI value within ± 2 % of their literature LRI value were accepted as a putatively identified substance. Furthermore, only compounds with a compound match rate of over 85% and that were present in at least two of the three biological replicates were retained as accepted compounds (Table S2, Supplementary Materials). The vast majority (>85%) of the compounds detected were contaminants (siloxanes) which are commonly emitted from the sample vials. Contaminants were characterised by removing all compounds detected in control vials from the vials containing samples prior to analysis. Compounds detected in the headspace of control vials were predominantly contaminants (siloxanes) from the vials. These materials represented >85% of all compounds detected in the samples and were subtracted from the list of compounds detected in the headspace of sample vials. Following putative identification, individual VOCs were assigned into chemical classes using mVOC 2.0 (Lemfack et al. 2018) and ClassyFire (Djoumbou Feunang et al. 2016).

Genomic identification of microbial isolates

Microbial isolates that passed pre-screening were subjected to preliminary taxonomic identification. Microbial DNA was extracted from liquid bacterial cultures and fungal agar plugs using a DNeasy UltraClean Microbial Kit (Qiagen, Germany) according to manufacturer's instructions. Methods for PCR amplification of the 16S rRNA gene region and ITS2 gene region are outlined in the Supplementary Methods. PCR products were cleaned using a Magnetic Bead PCR Cleanup Kit (Geneaid Biotech Ltd., Taiwan) and sequenced at the Lincoln University DNA Sequencing facility (Lincoln, New Zealand). Following sequencing, low quality ends were trimmed and forward and reverse reads were aligned using UniPro UGENE (version 36.0) software (Okonechnikov et al. 2012). Microbial isolates were identified to genus level based on the identity of their closest match from the NCBI BLAST rRNA and ITS database (GenBank). Bacterial 16S rRNA and fungal ITS2 gene regions were deposited in the NCBI database under the GenBank accession numbers shown in Table 1. To compare the relatedness of the 16S rRNA/ITS2 gene region sequences, multiple sequence alignments were performed by progressive pairwise alignment using Geneious Prime 2020.2.4 (https://www.geneious.com). Parameters for Geneious alignment were set to default. To build phylogenetic trees, the Neighbour Joining tree build method was selected using Tamura-Nei genetic distance models and bootstrap resampling.

RESULTS

Genomic identification of isolates

From the 11 bacterial isolates identified to significantly inhibit *P. agathidicida* mycelial growth in dual culture bioassays, nine were identified as belonging to the genus *Burkholderia*, one was identified as a *Paraburkholderia*, and one as *Pseudomonas*. All nine fungal isolates that significantly inhibited *Phytophthora agathidicida* in dual culture bioassays were identified to belong to the genus *Penicillium* (Table 1). Figures S3 and S4 (Supplementary Materials) show the phylogenetic relationships between the bacterial and fungal isolates displayed in Table 1.

In vitro bioassays

Dual culture bioassays

When tested using dual culture bioassays, all fungal and bacterial isolates that passed pre-screening significantly reduced *P. agathidicida* mycelial growth compared with *P. agathidicida* only control plates (p-value < 0.001, Fig. 1 and Fig. 2).

Fungal isolates with the highest mean mycelial inhibition (MI, %) were *Penicillium* ks20_f18 (58.28 ± 1.55), *Penicillium* ks20_f30 (57.27 ± 1.20), and *Penicillium* ks20_f52 (55.27 ± 1.26). Bacterial isolates with the highest mean MI values (%) were *Burkholderia* ks20_b71 (60.88 ± 3.22), *Burkholderia* ks20_b12 (59.91 ± 1.84), and *Burkholderia* ks20_b69 (59.78 ± 1.37). Images of fungal and bacterial dual culture bioassays are shown in Fig. S5 and Fig. S6, Supplementary Materials. The results of the bioassays and the statistical tests are provided in more detail in Tables S3 and S4, Supplementary Materials. Several isolates of *Penicillium*

Table 1 Identification of bacterial and fungal isolates that significantly inhibited *Phytophthora agathidicida* in dual culture bioassays. The taxonomic assignment of each bacterial and fungal isolate was performed to genus level based on the identity of their closest match when their 16S rRNA/ITS2 gene region sequences were searched in the NCBI BLAST database.

Identified isolate	Accession number	Closest match	Expect value	% match
Burkholderia ks20_b4	MW041148	*B. catarinensis* (NR_153664.1)	0.0	99.15
Burkholderia ks20_b8	MW040830	*B. catarinensis* (NR_153664.1)	0.0	98.44
Paraburkholderia ks20_b72	MW040841	*Paraburkholderia metalliresistens* (NR_118054)	0.0	97.51
Burkholderia ks20_b9	MW040831	*B. catarinensis* (NR_153664.1)	0.0	97.87
Burkholderia ks20_b69	MW041147	*B. catarinensis* (NR_153664.1)	0.0	97.62
Burkholderia ks20_b71	MW040834	*B. catarinensis* (NR_153664.1)	0.0	99.13
Burkholderia ks20_b31	MW040833	*B. catarinensis* (NR_153664.1)	0.0	98.50
Burkholderia ks20_b16	MW041149	*B. catarinensis* (NR_153664.1)	0.0	99.15
Burkholderia ks20_b74	MW040835	*B. catarinensis* (NR_153664.1)	0.0	98.91
Burkholderia ks20_b12	MW040832	*B. catarinensis* (NR_153664.1)	0.0	98.54
Pseudomonas ks20_b65	MW040836	*Pseudomonas helleri* (NR_148763.1)	0.0	97.86
Penicillium ks20_ f10	MW040805	*Penicillium thomii* (NR_077159.1)	2e-173	99.11
Penicillium ks20_ f20	MW040810	*Penicillium bialowiezense* (NR_165994.1)	1e-165	96.58
Penicillium ks20_ f52	MW040812	*Penicillium montanense* (NR_138270.1)	2e-163	98.76
Penicillium ks20_ f54	MW040813	*Penicillium thomii* (NR_077159.1)	8e-167	97.66
Penicillium ks20_ f14	MW040806	*Penicillium daejeonium* (NR_158791.1)	1e-169	98.23
Penicillium ks20_ f15	MW040807	*Penicillium kiamaense* (NR_137899)	2e-168	99.69
Penicillium ks20_ f18	MW040808	*Penicillium malachiteum* (NR_120271.1)	0.0	99.15
Penicillium ks20_ f19	MW040809	*Penicillium montanense* (NR_138270.1)	5e-174	98.55
Penicillium ks20_ f30	MW040811	*Penicillium montanense* (NR_138270.1)	1e-175	98.29

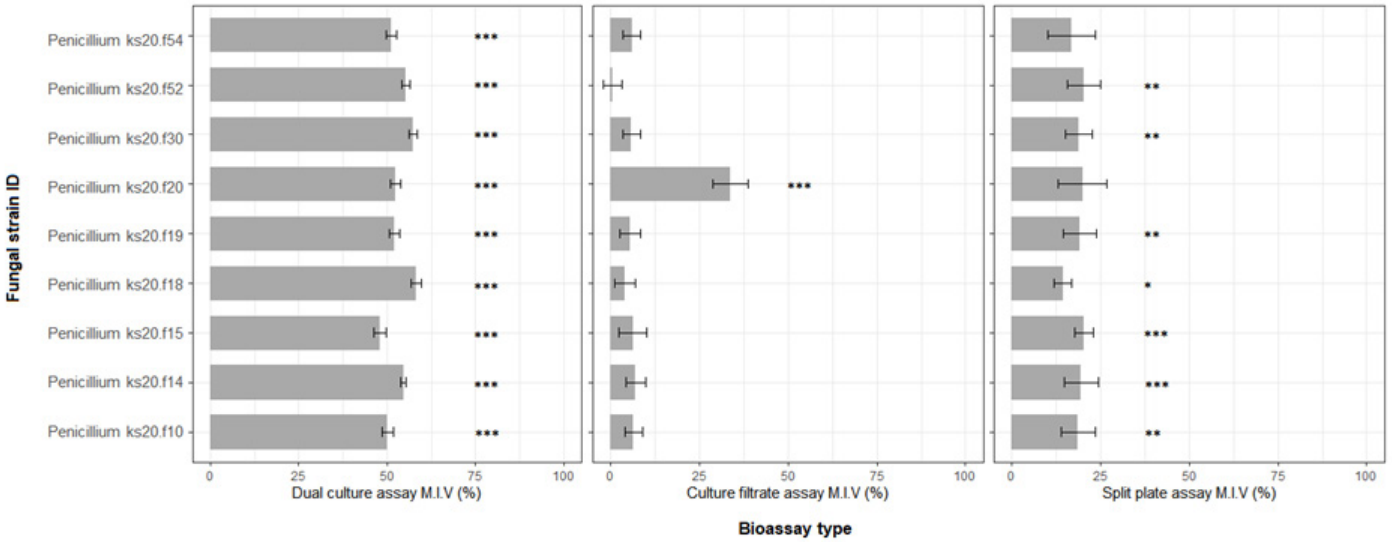

Figure 1 The mean ± SE mycelial inhibition values (MIV %) of the in vitro bioassays used to assess the inhibition of fungal isolates against *Phytophthora agathidicida*. Student T tests were used to identify isolates with significantly higher MIV compared to controls (* is *p*-value < 0.05, ** is *p*-value < 0.01 and *** is *p*-value < 0.001).

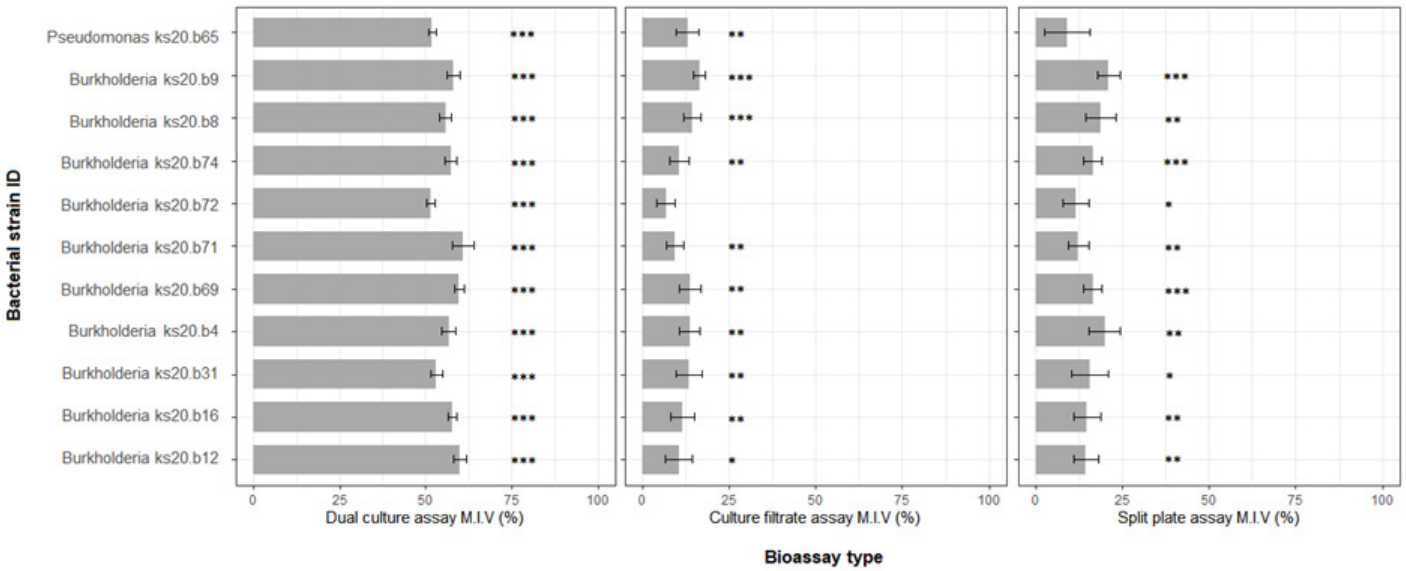

Figure 2 The mean ± SE mycelial inhibition values (MIV %) of the in vitro bioassays used to assess the inhibition of bacterial isolates against *Phytophthora agathidicida*. Student T tests were used to identify isolates with significantly higher MIV compared to controls (* is *p*-value < 0.05, ** is *p*-value < 0.01 and *** is *p*-value < 0.001).

overgrew the mycelium of *P. agathidicida* in dual culture (Fig. S7, Supplementary Materials).

Culture filtrate bioassays

The culture filtrates of only one fungal isolate, *Penicillium* ks20_f20, significantly reduced the mycelial growth of *Phytophthora agathidicida* when compared with controls (mean MI: 33.72 ± 4.95%; *p*-value < 0.001, Fig. 1). As well as inhibiting mycelial growth, the culture filtrates of *Penicillium* ks20_f20 noticeably reduced the mycelial density of *Phytophthora agathidicida* (Fig. 3).

Burkholderia ks20_b72 was the only bacterial isolate that did not significantly reduce *P. agathidicida* mycelial growth (*p*-value > 0.05). As shown in Fig. 2, the culture filtrates of all other 10 bacterial isolates significantly reduced *P. agathidicida* mycelial growth compared to controls (*p*-value < 0.05). Bacterial isolates whose filtrates had the highest mean MI value (%) were *Burkholderia* ks20_b9 (16.43 ± 1.71), *Burkholderia* ks20_b8 (14.40 ± 2.50), and *Burkholderia* ks20_b69 (13.69 ± 3.19).

Split plate bioassays

When tested using split plate bioassays, seven *Penicillium* isolates significantly reduced *Phytophthora agathidicida* mycelial growth compared with controls (*p*-value < 0.05, Fig. 1). Isolates with the highest mean MI values (%) were *Penicillium* ks20_f15 (20.26 ± 2.64) and *Penicillium* ks20_f52 (20.33 ± 4.76). *Penicillium* ks20_f20 and *Penicillium* ks20_

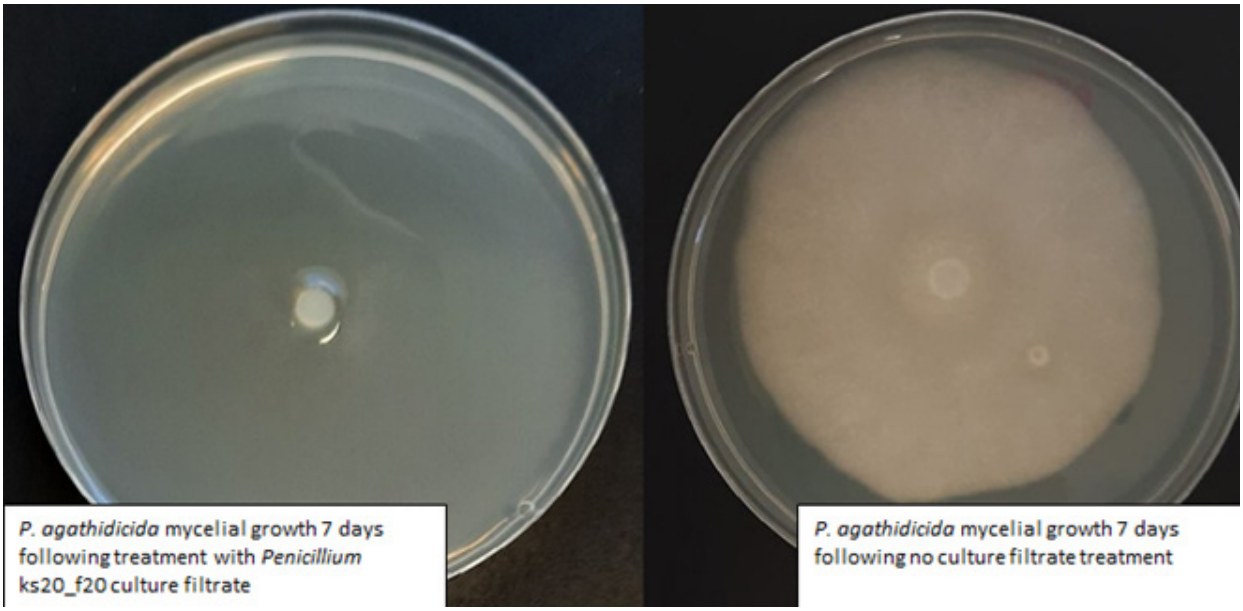

Figure 3 The mycelial growth of *Phytophthora agathidicida* on day 7 following culture filtrate treatment with *Penicillium* ks20_f20 versus the mycelial growth of *Phytophthora agathidicida* on day 7 that had no culture filtrate treatment.

f54 did not significantly reduce *Phytophthora agathidicida* mycelial growth compared to controls (p-value > 0.05).

Except for *Pseudomonas* ks20_b65, all bacterial isolates significantly reduced *Phytophthora agathidicida* mycelial growth compared to controls when tested using split plate bioassays (p-value < 0.05, Fig. 2). The highest mean MI values (%) were found for *Burkholderia* ks20_b9 (21.03 ± 3.17) and *Burkholderia* ks20_b4 (19.87 ± 4.49).

VOC profiles of microbial isolates

From the results of the HS SPME-GC-MS analysis, we putatively identified five volatile organic compounds (VOCs) produced by *P. agathidicida*, which were 2-phenylethanol (0.65 ± 0.07%); methyl salicylate (0.31 ± 0.14%); 2,2,4-trimethyl-1,3-pentanediol di-isobutyrate (0.35 ± 0.02%); and 2,5-di-tert-butyl-1,4-benzoquinone (0.42 ± 0.09%). None of these VOCs were putatively identified to be produced by the fungal or bacterial isolates subsequently analysed (Fig. 4 and Fig. 5). Based on these results, no VOCs produced by the fungal and bacterial isolates were also produced by *P. agathidicida*.

Across all the fungal isolates analysed; 32 different VOCs were putatively identified (Fig. 4). These covered six chemical classes – sesquiterpenoids, monoterpenoids, ketones, hydrocarbons, fatty alcohols, and benzenoids (Fig. S8, Supplementary Materials). We did not detect any identifiable VOCs from *Penicillium* ks20_f20. This finding is consistent with the results of the split plate bioassays, which found that this isolate did not significantly inhibit *Phytophthora agathidicida* mycelial growth (Fig. 1). Although *Penicillium* ks20_f54 did not significantly inhibit *P. agathidicida* mycelial growth when tested using split plate bioassays (Fig. 1), three identifiable VOCs produced by this isolate were detected(Fig. 4). Two of these VOCs, 3-octanone and 1-octen-3-ol, were also produced by *Penicillium* ks20_f30. Therefore, these two VOCs are unlikely to be responsible for the mycelial inhibition observed by *Penicillium* ks20_f30.

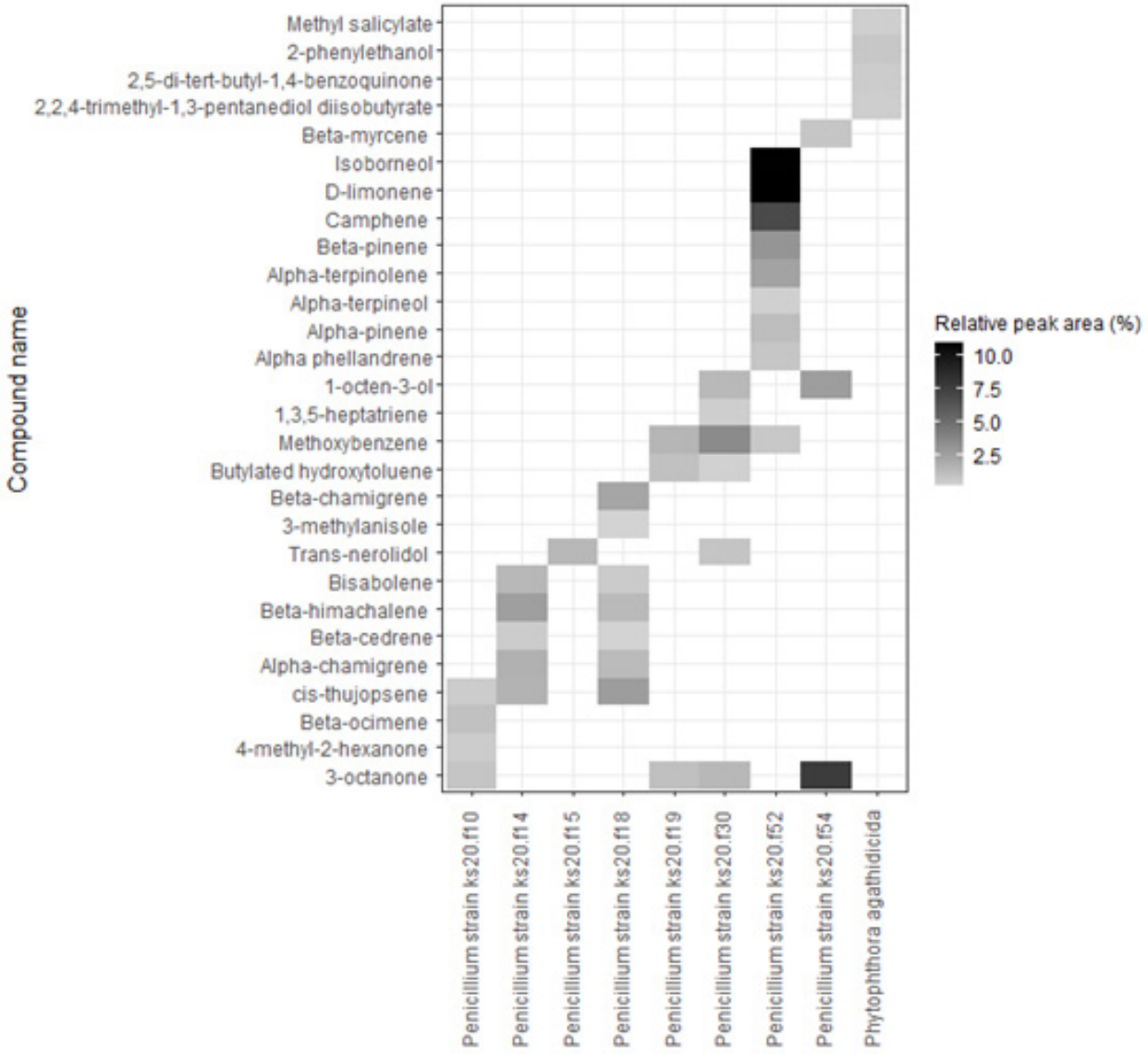

Figure 4 The peak areas (%) of the putatively identified VOCs released by the *Penicillium* isolates and *Phytophthora agathidicida* (which was functioning as a control) relative to the total peak area for all compounds. For all samples, siloxane contaminants from the vials constituted most of the VOCs detected in the headspace (not shown). *Penicillium* ks20_f20 is not shown in this figure as no identifiable VOCs were detected from this isolate.

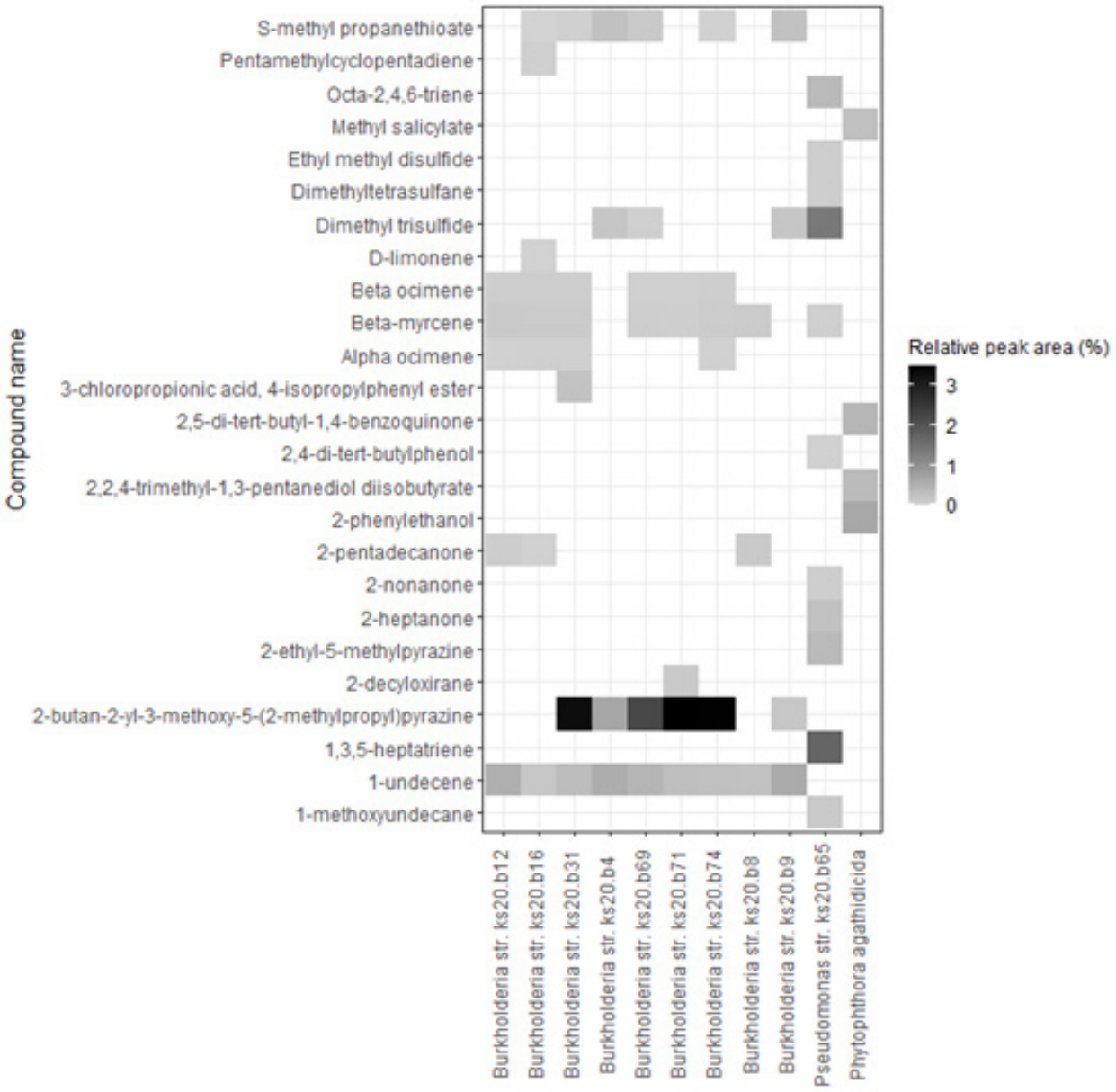

Figure 5 The peak areas (%) of the putatively identified VOCs released by the *Burkholderia* isolates and *Phytophthora agathidicida* (which was functioning as a control) relative to the total peak area for all compounds. For all samples, siloxane contaminants from the vials constituted most of the VOCs detected in the headspace (not shown). *Paraburkholderia* ks20_b72 is not shown on this figure as no identifiable VOCs were detected from this isolate.

Across all the bacterial isolates analysed; 29 VOCs were putatively identified (Fig. 5). These covered 13 chemical classes – alkenes, benzenoids, epoxides, fatty acyls, hydrocarbons, ketones, monoterpenoids, organic disulphides, organic oxides, organic trisulfides, pyrazines, sesquiterpenoids, and thiocarboxylic acids (Fig. S8, Supplementary Materials). The alkene compound 1-undecene was putatively identified to be produced in the head space from all the *Burkholderia* isolates. Additionally, the pyrazine compound 2-butan-2-yl-3-methoxy-5-(2-methylpropyl) pyrazine had a high relative peak area in several of the *Burkholderia* isolates (Fig. 5). Based on our methods, we were not able to identify any compounds produced by *Paraburkholderia* ks20_b72. Except for beta-myrcene and dimethyl trisulphide, no identifiable VOCs produced by *Pseudomonas* ks20_b65 were produced by the other *Burkholderia* isolates (Fig. 5). *Pseudomonas* ks20_b65 did not significantly inhibit *Phytophthora agathidicida* mycelial growth in split plate bioassays, thus these results suggest that this strain is not inhibiting *P. agathidicida* through the production of inhibitory VOCs.

DISCUSSION

When tested using dual culture bioassays, microbial isolates belonging to the genera *Penicillium*, *Burkholderia*, *Paraburkholderia*, and *Pseudomonas* significantly inhibited the mycelial growth of *Phytophthora agathidicida*. It is likely that multiple isolates of the same species were obtained but further, more detailed genomic analysis and morphological

identification are required in order to assign isolates to species level and this work was outside the scope of the current study. Several isolates inhibited *P. agathidicida* in culture filtrate and split plate bioassays, which suggests that the production of diffusible and volatile compounds may be factors contributing to inhibition. However, the inhibition of *P. agathidicida* was much lower in culture filtrate and split plate bioassays than in dual culture bioassays. A similar finding was observed by Elshafie et al. (2012) who found a greater inhibition of *Phytophthora cactorum* by *Burkholderia gladioli* pv. *agaricicola* in dual culture bioassays compared to culture filtrate treatments. The greater inhibition observed in dual culture bioassays may be due to the synergistic effects of multiple modes of antagonism acting against *P. agathidicida* (i.e. microbial competition combined with the production of anti-microbial compounds), rather than a single mode of action being responsible for most of the observed inhibition.

Burkholderia isolates display strong inhibition towards *P. agathidicida*

In each of the three bioassays used to test for inhibition, all nine *Burkholderia* isolates significantly inhibited the mycelial growth of *P. agathidicida*. *Burkholderia* species have previously been reported to antagonise a wide range of pathogens, including *Phytophthora* species (Elshafie et al. 2012; Sophearetth et al. 2013; Kong et al. 2020). When screened using dual culture bioassays, there was a clear zone of inhibition present on agar and no isolates came into direct contract with *P. agathidicida*. Therefore, it is unlikely that inhibition was a result of direct mycoparasitism or microbial competition.

All *Burkholderia* isolates significantly inhibited the growth of *P. agathidicida* in split plate and culture filtrate bioassays, albeit at a lower level of inhibition than in dual culture. Despite inhibition being lower in the culture filtrate bioassays, these results support that compounds released by the bacterial isolates may be contributing to inhibition. This notion is supported by the clear zones of inhibition observed in the dual culture bioassays. Future research may wish to identify if inhibitory compounds are being released by the bacteria and diffusing through the agar. The production of secondary metabolites such as siderophores, antibiotics, hydrolytic enzymes, and biosurfactants are known to contribute to the suppression of pathogenic fungi by bacteria (Neeraja et al. 2010). Furthermore, *Burkholderia* species have been demonstrated to produce a wide range of anti-microbial secondary metabolites (Li et al. 2002; Vial et al. 2007; Depoorter et al. 2016). Further research that characterises the secondary metabolites produced by the *Burkholderia* isolates, both on solid agar and in culture filtrate form, is required to understand the mechanisms driving inhibition of *P. agathidicida* by these strains.

The results of the culture filtrate bioassays did not provide any evidence that *Paraburkholderia* ks20_b72 produced compounds that are inhibitory towards *Phytophthora agathidicida*. However, this isolate was observed to significantly inhibit *P. agathidicida* in dual culture and split plate bioassays. Similarly, despite being found to produce a diverse range of VOCs by HS SPME-GC-MS analysis, *Pseudomonas* ks20_b65 did not significantly inhibit *P. agathidicida* when tested using split plate bioassays. This contrasts with previous research by Hunziker et al. (2015) and De Vrieze et al. (2015), who identified that *Pseudomonas* isolates can produce VOCs with anti-*Phytophthora* activities. *Pseudomonas* ks20_b65 significantly inhibited *Phytophthora agathidicida* in dual culture and culture filtrate bioassays, suggesting the production of other anti-microbial compounds may be a potential mode of action by this strain. Previous studies have identified strains of *Pseudomonas* that produce a wide range of inhibitory compounds active against phytopathogens such as siderophores, broad-spectrum antibiotics, lipopeptides, polyketides, and biosurfactants (Nielsen et al. 2006; Caulier et al. 2018; Arora et al. 2008; Tran et al. 2007). Further research is needed to identify the modes of action contributing to the inhibition of *P. agathidicida* by *Pseudomonas* ks20_b65 and *Paraburkholderia* ks20_b72 observed in dual culture.

Penicillium isolates were variable in their inhibition of *P. agathidicida*

Several isolates of *Penicillium* overgrew the mycelium of *P. agathidicida* in dual culture, which suggests that these isolates may have inhibited *P. agathidicida* by having a more competitive growth rate on agar which physically limited the growth of *P. agathidicida* (Bunbury-Blanchette & Walker 2019). These results align with the findings of Byers et al. (2020), which found that the relative abundance of *Penicillium* was significantly higher in soils associated with healthy, asymptomatic kauri compared to symptomatic kauri expressing the symptoms of dieback disease. For biological control agents whose main mode of action is through competition, sustaining high population levels in the soil environment is essential for them to suppress target pathogens (Alabouvette et al. 2006). Quite often, microbial antagonists fail to confer disease suppression when applied in the field as they cannot compete with the resident soil microbiota to successfully establish within the rhizosphere (Expósito et al. 2017). *Penicillium* species are well-adapted and highly competitive members of the soil environment and have been demonstrated to antagonise a variety of plant pathogens (Nicoletti & De Stefano 2012). Therefore, the identification of *Penicillium* isolates demonstrating inhibition towards *Phytophthora agathidicida* is promising in regard to their practical application in the field. Biological control agents (BCAs) with non-chemical modes of action (i.e. nutrient competition) have a lower likelihood of pathogen resistance developing. In addition, their eco-toxicological risk and associated risk assessments required to permit their application are much lower as they are inhibiting pathogens through general ecological processes rather than the production of anti-microbial compounds (Köhl et al. 2019).

The culture filtrates of *Penicillium* ks20_f20 strongly inhibited *Phytophthora agathidicida* and this isolate produced a clear zone of inhibition in dual culture. However, the limitations of quantifying inhibition by measuring the reduction in the mycelial growth of *P. agathidicida* was evident when assessing the inhibitory potential of this strain, as measurements of mycelial diameter did not

reflect the large reductions in the mycelial density of *P. agathidicida*. Future research should measure the impact of each *P. agathidicida* isolate on sporangia production and zoospore release to better quantify their inhibitory potential. Nonetheless, our results support that *Penicillium* ks20_f20 produced anti-microbial compounds inhibitory towards *Phytophthora agathidicida*. As with isolates of *Burkholderia*, more research is required to characterise these compounds with potential anti-microbial action, as it is important to fully understand their mode of action before they can be appropriately and effectively applied in the field for biocontrol (Spadaro & Gullino 2005).

Contribution of microbial VOCs to the inhibition of *P. agathidicida*

All the *Burkholderia* isolates inhibited the growth of *P. agathidicida* in split plate bioassays, suggesting they may be releasing VOCs with inhibitory properties against *P. agathidicida*. The putatively identified compound 1-undecene was produced by all the *Burkholderia* isolates. This compound has previously been found to inhibit the mycelial growth, sporangia formation and zoospore release of *Phytophthora infestans* (Hunziker et al. 2015). Another putatively identified VOC emitted by several of the *Burkholderia* isolates was 2-butan-2-yl-3-methoxy-5-(2-methylpropyl) pyrazine. Although this compound has not been studied for its anti-microbial properties, other pyrazine VOCs (i.e. 2, 5-dimethyl pyrazine and 2-methoxy-3-methyl pyrazine) have previously been identified to inhibit the growth of *Phytophthora infestans* and *P. capsici* (Munjal et al. 2016; Lazazzara et al. 2017). Several *Burkholderia* isolates were identified to produce the two monoterpenoid compounds alpha ocimene and beta ocimene. Tenorio-Salgado et al. (2013) identified ocimene compounds to be produced by isolates of *Burkholderia tropica* that inhibited several plant pathogens, although inhibition was not tested for *Phytophthora*. In addition, ocimene compounds were identified as components of plant essential oils that were able to significantly inhibit the mycelial growth of *Phytophthora capsici, P. drechsleri*, and *P. melonis* (Amini et al. 2016).

Most *Penicillium* isolates isolated in this study significantly inhibited *P. agathidicida* in split plate bioassays. Many of the VOCs produced by these isolates were putatively identified as terpenoids, ketones, and benzenoids. However, the scientific knowledge regarding the inhibitory roles of VOCs produced by *Penicillium* against fungal plant pathogens is limited as *Penicillium* species have been more thoroughly researched for their production of anti-bacterial compounds (Rouissi et al. 2013). In addition, unlike the isolates of *Burkholderia*, no VOCs were identified to be consistently produced by the *Penicillium* isolates that inhibited *Phytophthora agathidicida*.

To characterise the VOC profile of each isolate, this study incubated each microbial antagonist in isolation (i.e. not in the presence of *P. agathidicida*) prior to HS SPME-GC-MS analysis. However, this did not allow us to detect novel VOCs which may be produced by the isolates when in the presence of *P. agathidicida*. When interacting under a shared environment, the production of microbial VOCs can mediate interspecies communication (Schmidt et al. 2015). Furthermore, the release of microbial VOCs with roles in antibiosis and signalling can serve as an important regulatory mechanism under resource competitive environments (Effmert et al. 2012). When exposed *P. agathidicida,* the VOCs released by each isolate may have been different to what was characterised when using our methods. Therefore, future research should aim to characterise the VOC profiles of each microbial antagonist when in the presence of *P. agathidicida*.

CONCLUSIONS

This study identified isolates belonging to the genera *Burkholderia* and *Penicillium* that exhibit different modes of action against *P. agathidicida*, and warrant further research as candidates for its biocontrol. As all isolates of *Burkholderia* were able to inhibit *P. agathidicida* mycelial growth without establishing direct contact, the mode of inhibition may be through production of diffusible and volatile inhibitory compounds. Many of the *Penicillium* isolates had a fast growth rate that restricted the growth of *Phytophthora agathidicida* on agar, suggesting that microbial competition is a likely mode of inhibition. Further research should assess the synergistic effects of these different microbial isolates, as combining different strains of microbial antagonists can often provide a higher level of disease suppression as multiple different modes of antagonism are acting against the target pathogen (Spadaro & Gullino 2005; De Vrieze et al. 2018).

HS SPME-GC-MS analysis putatively identified several VOCs, such as 1-undecene, as being produced by many of the *Burkholderia* isolates. Further research is required to more accurately identify these VOCs by comparing their retention times and mass spectra with pure reference standards of the respective compounds. Furthermore, the direct effects of these VOCs in their pure form on the growth of *P. agathidicida* need to be assessed to identify if they are responsible for the inhibition observed in this study. The cultures filtrates of all *Burkholderia* isolates and one *Penicillium* isolate inhibited the growth of *Phytophthora agathidicida*. The compounds present in these culture filtrates need to be characterised so that their direct impacts on the growth of *P. agathidicida* mycelia can be assessed.

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ADDITIONAL FILES

Supplementary Methods
Supplementary Materials

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Supplementary Methods

S1. Bacterial and fungal selective agar recipes

Bacterial selective agar, per litre

- 28 g nutrient agar (Oxoid Ltd., UK)
- 200 mg of pentachloronitrobenzene (PCNB) (Sigma Aldrich, Missouri USA)
- 400 µL of pimaricin (Sigma Aldrich, Missouri USA)
- 50 mg of hymexazol (Tokyo Chemical Industry Co., Ltd, Japan)

Fungal selective agar, per litre

- 39 g of potato dextrose agar (Oxoid Ltd., UK)
- 250 mg of chloramphenicol (Sigma Aldrich, Missouri USA)
- 90 mg of streptomycin sulphate (Sigma Aldrich, Missouri USA)
- 50 mg of hymexazol (Tokyo Chemical Industry Co., Ltd, Japan)

S2. PCR conditions for Sanger sequencing the 16S rRNA/ITS gene region of microbial isolates

The 16S rRNA gene region was amplified using the forward and reverse primers 341F/785R (Thijs et al. 2017) and the ITS2 gene region was amplified using the forward and reverse primers ITS3/ITS4 (Op De Beeck et al. 2014) to obtain preliminary genetic information. Each 25 µL PCR reaction consisted of 0.5 µL of forward and reverse primers at a 10 µM concentration, 4 µL HOT FIREPol Blend Master Mix (Solis Biodyne, Estonia; https://www.solisbiodyne.com/EN/product/name=HOT-FIREPol-Blend-Master-Mix-Ready-to-Load&catno=04-25-00S25), 19 µL of nuclease free water (Invitrogen, Massachusetts USA), and 1 µL of DNA. PCR thermocycling conditions were 95°C for 12 minutes, followed by 30 cycles of 95°C for 15 seconds, 55°C for 30 seconds, 72°C for 30 seconds and a final extension phase of 75°C for 5 minutes. PCR products were run on 1% agarose gel at 100 V for 15 minutes to confirm amplification of the desired band.

References: Supplementary Methods

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Supplementary Materials

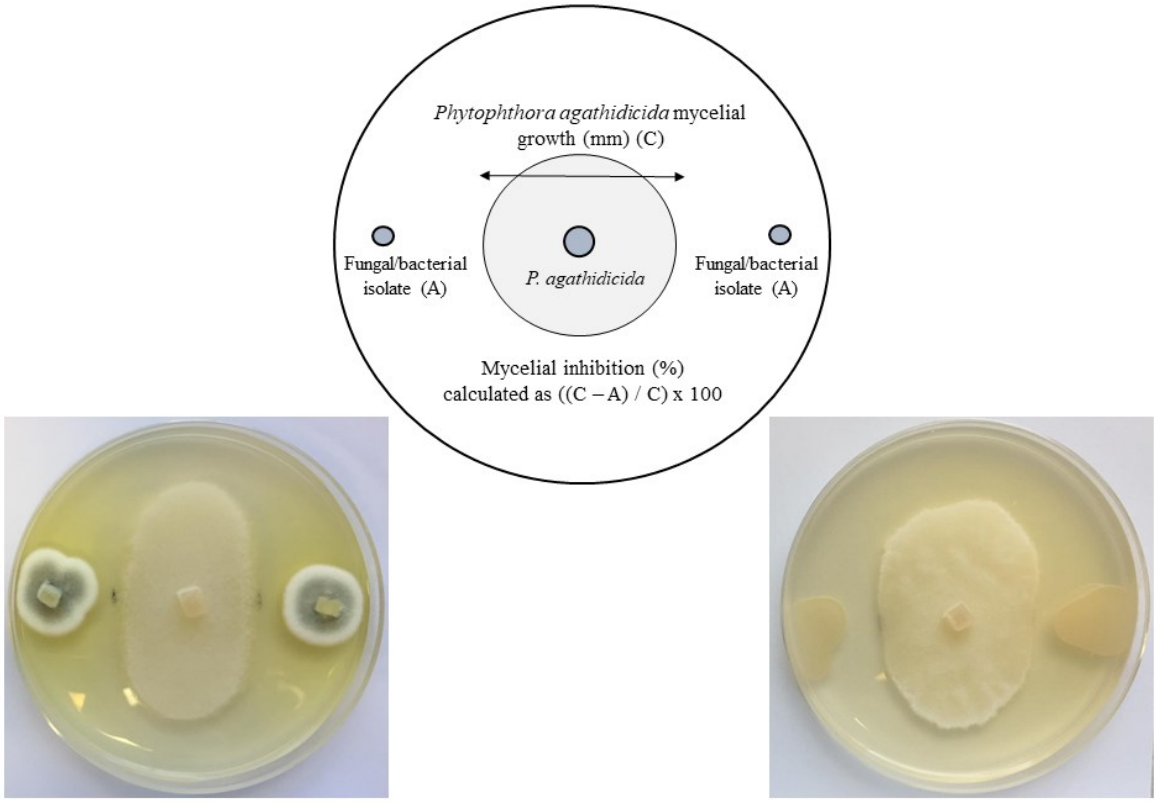

Figure S1 The experimental set up of the dual culture bioassays. Dual culture bioassays were used as the primary form of bioassay to screen fungal and bacterial isolates for their potential to inhibit *Phytophthora agathidicida*.

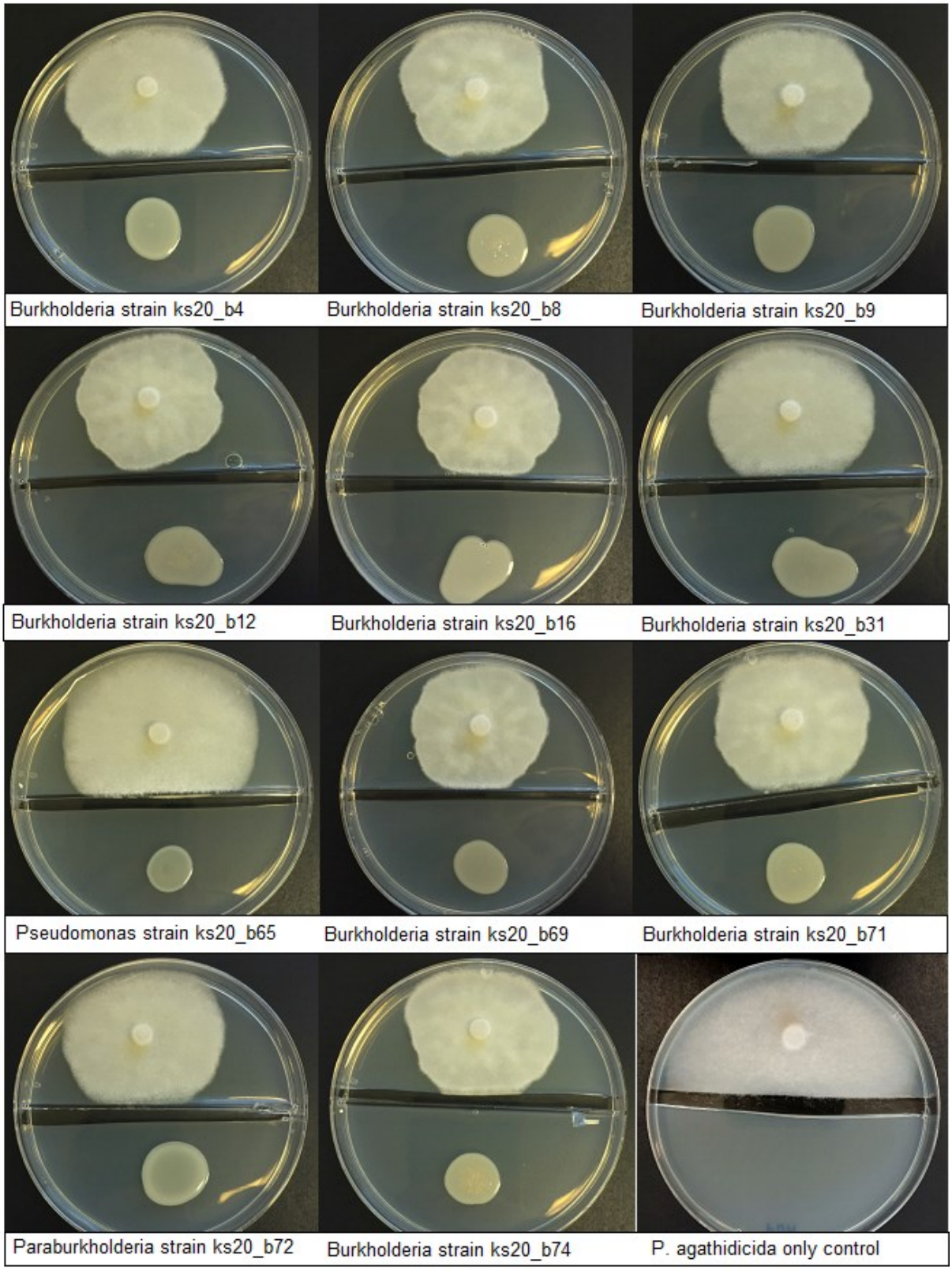

Figure S2 Example images of the bacterial split plate bioassays used to assess whether the microbial isolates may be producing VOCs which are inhibiting the mycelial growth of *Phytophthora agathidicida*.

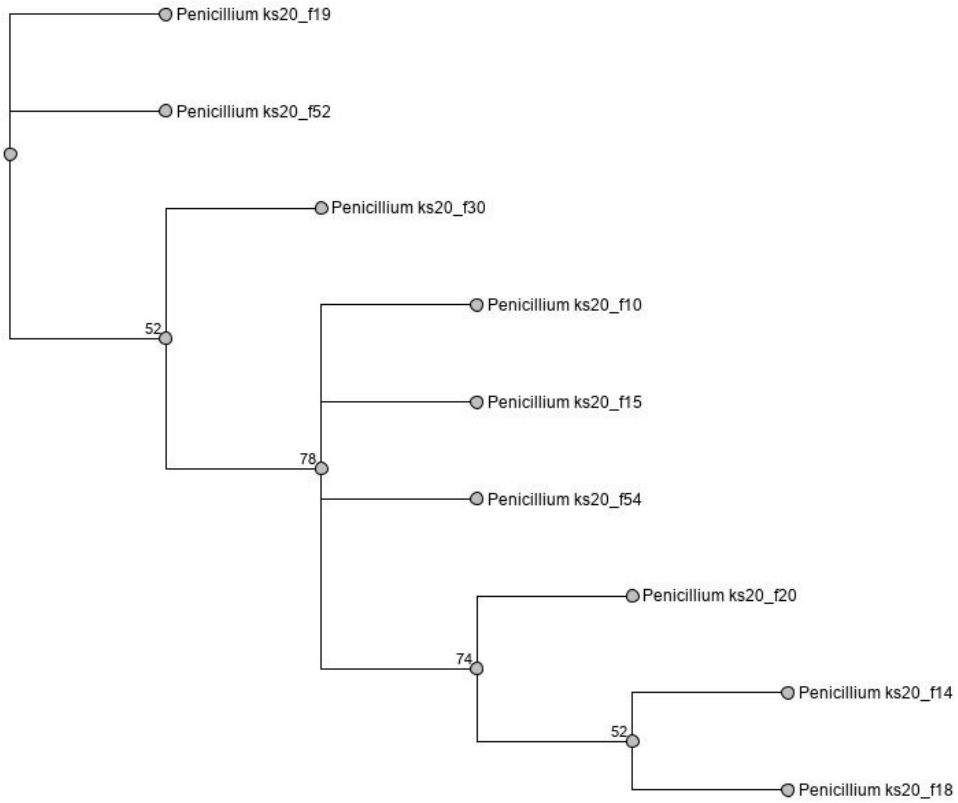

Figure S3 The phylogenetic relationships of the *Penicillium* strains isolated in this study, based on the alignment of their ITS2 gene region sequences. The phylogenetic tree was constructed using the Neighbour Joining tree build method with Tamura Nei selected as the genetic distance model and resampling performed using bootstrapping. Bootstrap values are shown as percentages next to the branches.

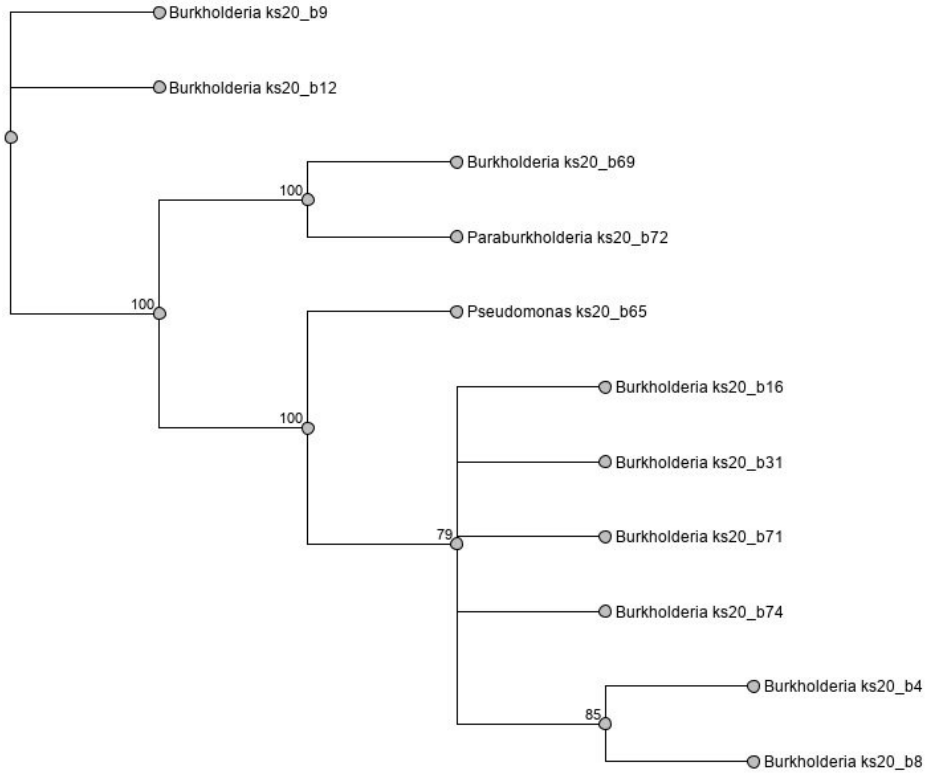

Figure S4 The phylogenetic relationships of the bacterial strains identified to inhibit *P. agathidicida,* based on the alignment of their 16S rRNA gene region sequences. Phylogenetic tree was constructed using the Neighbour Joining tree build method with Tamura Nei selected as the genetic distance model and resampling performed using bootstrapping. Bootstrap values are shown as percentages next to the branches.

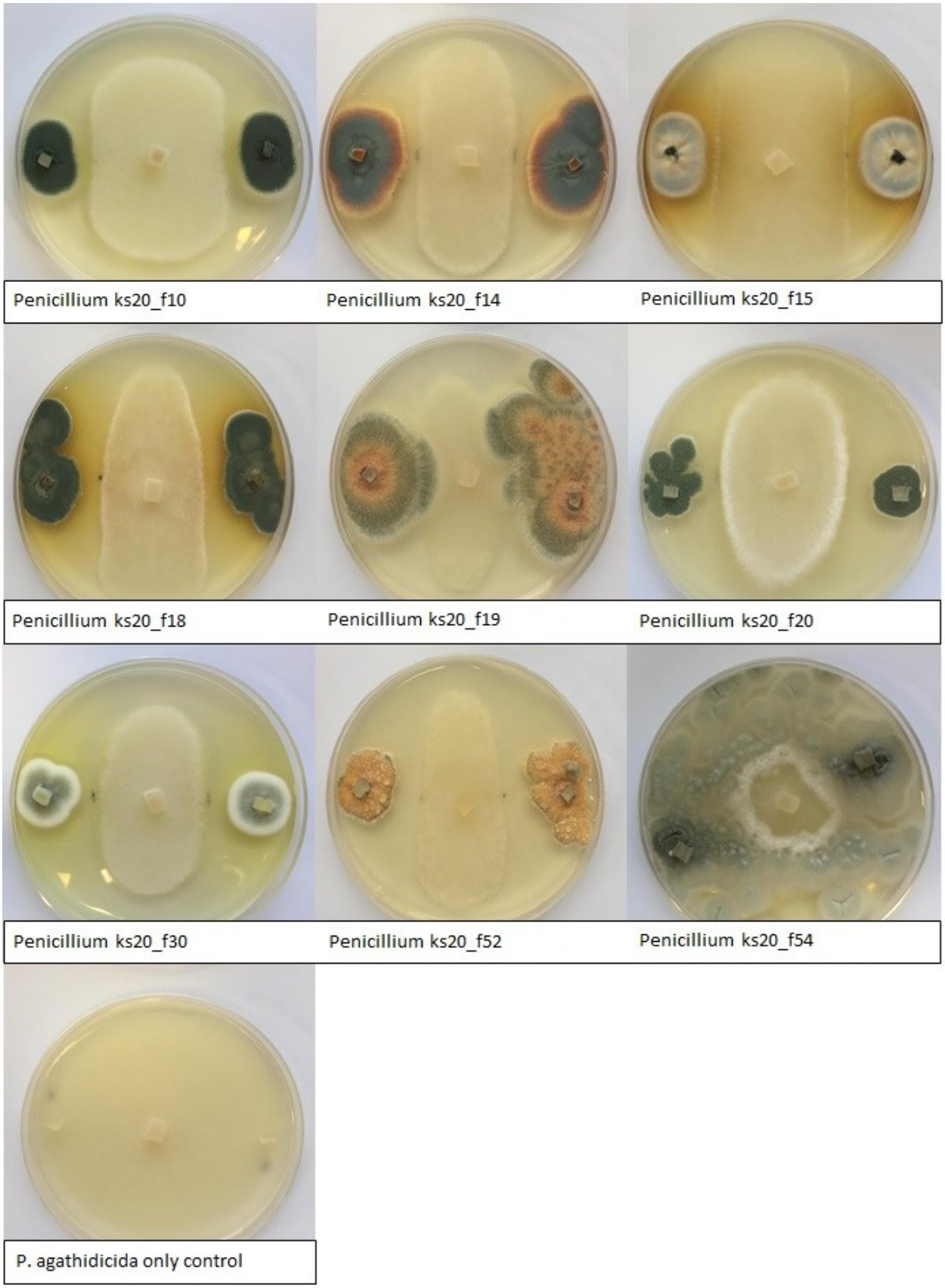

Figure S5 The fungal isolates that significantly reduced *P. agathidicida* mycelial growth compared with *P. agathidicida* only controls in dual culture bioassays.

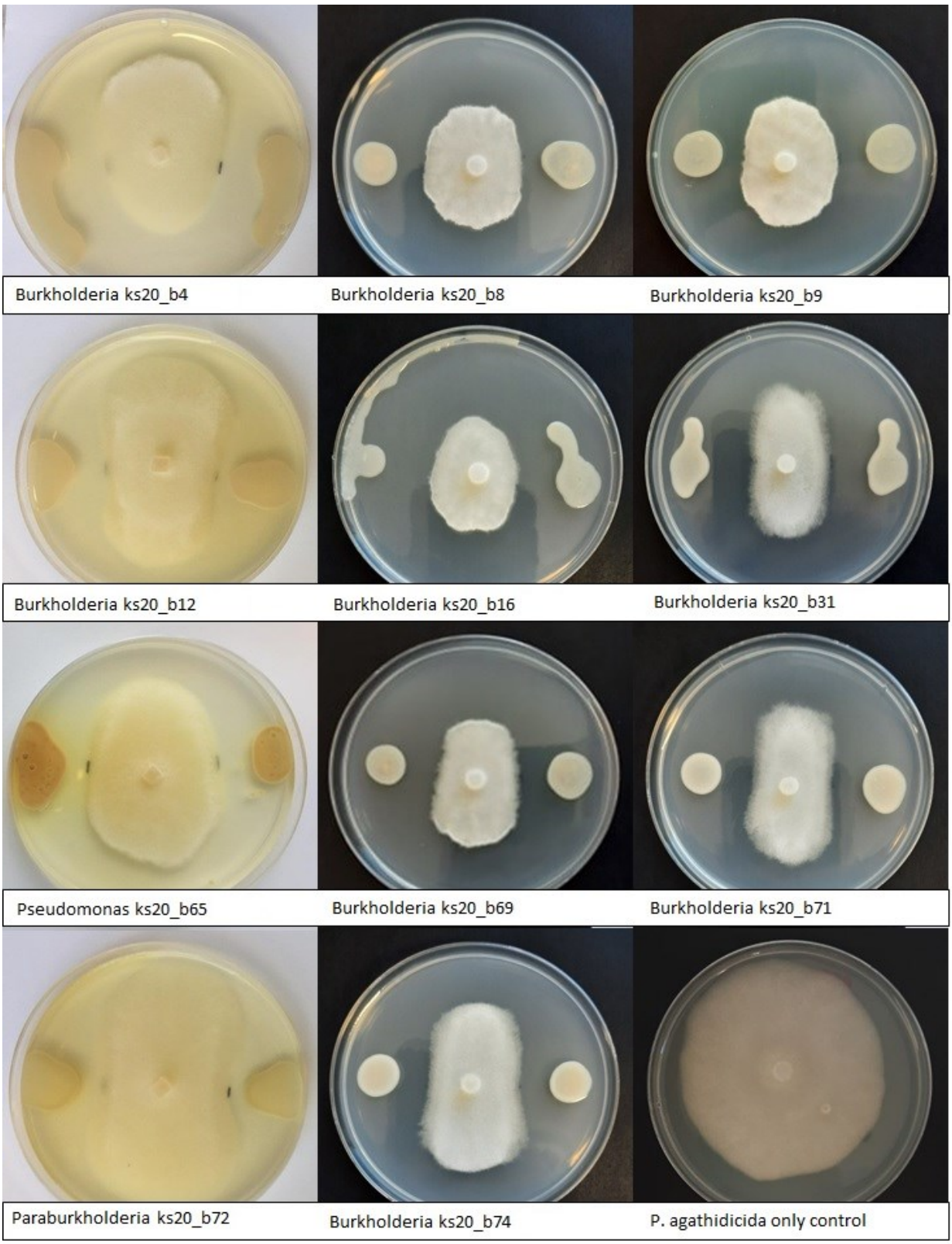

Figure S6 The bacterial isolates that significantly reduced *P. agathidicida* mycelial growth compared with *P. agathidicida* only controls in dual culture bioassays.

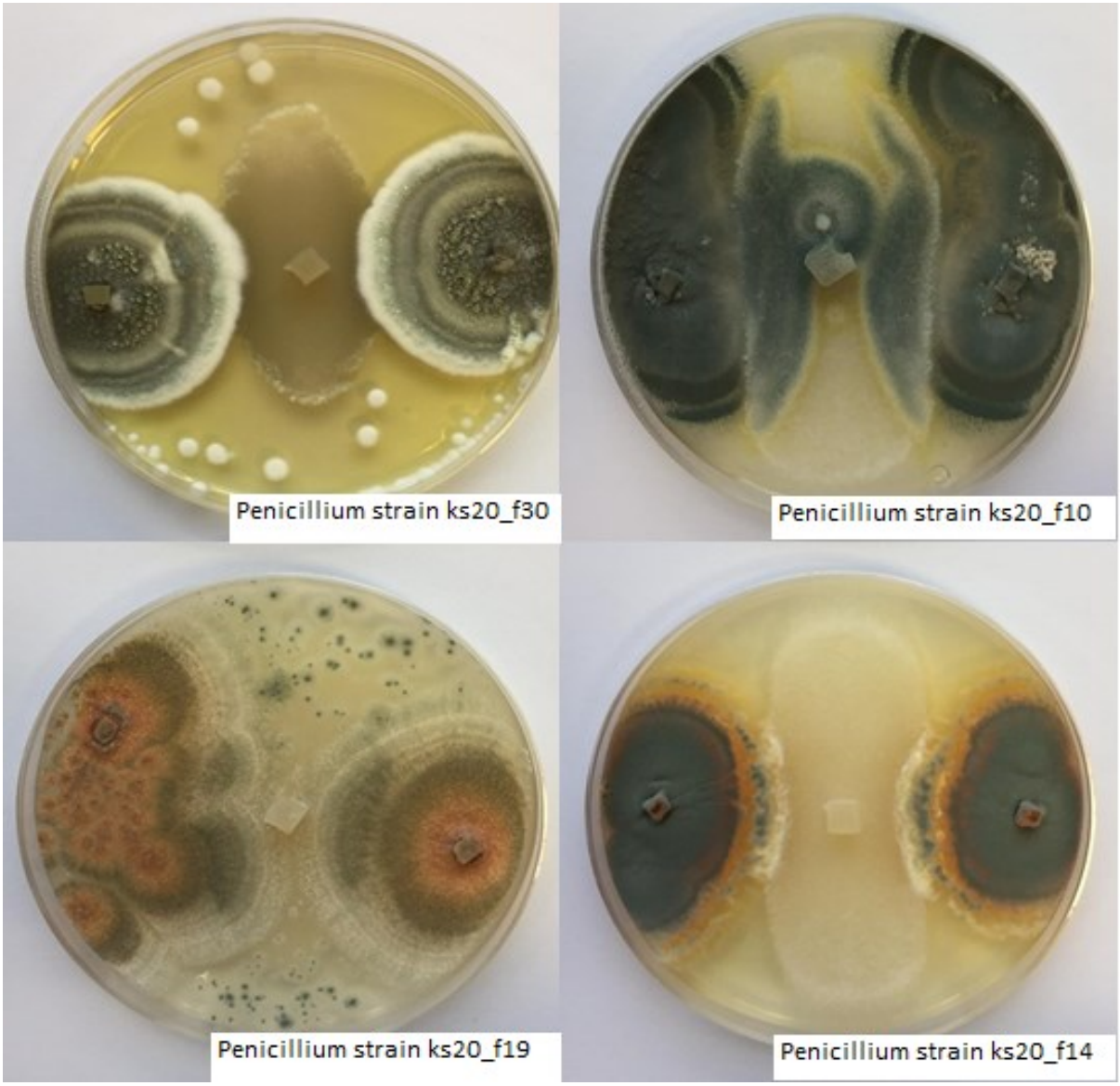

Figure S7 Fungal isolates which grew over *P. agathidicida* mycelium when screened using dual culture bioassays.

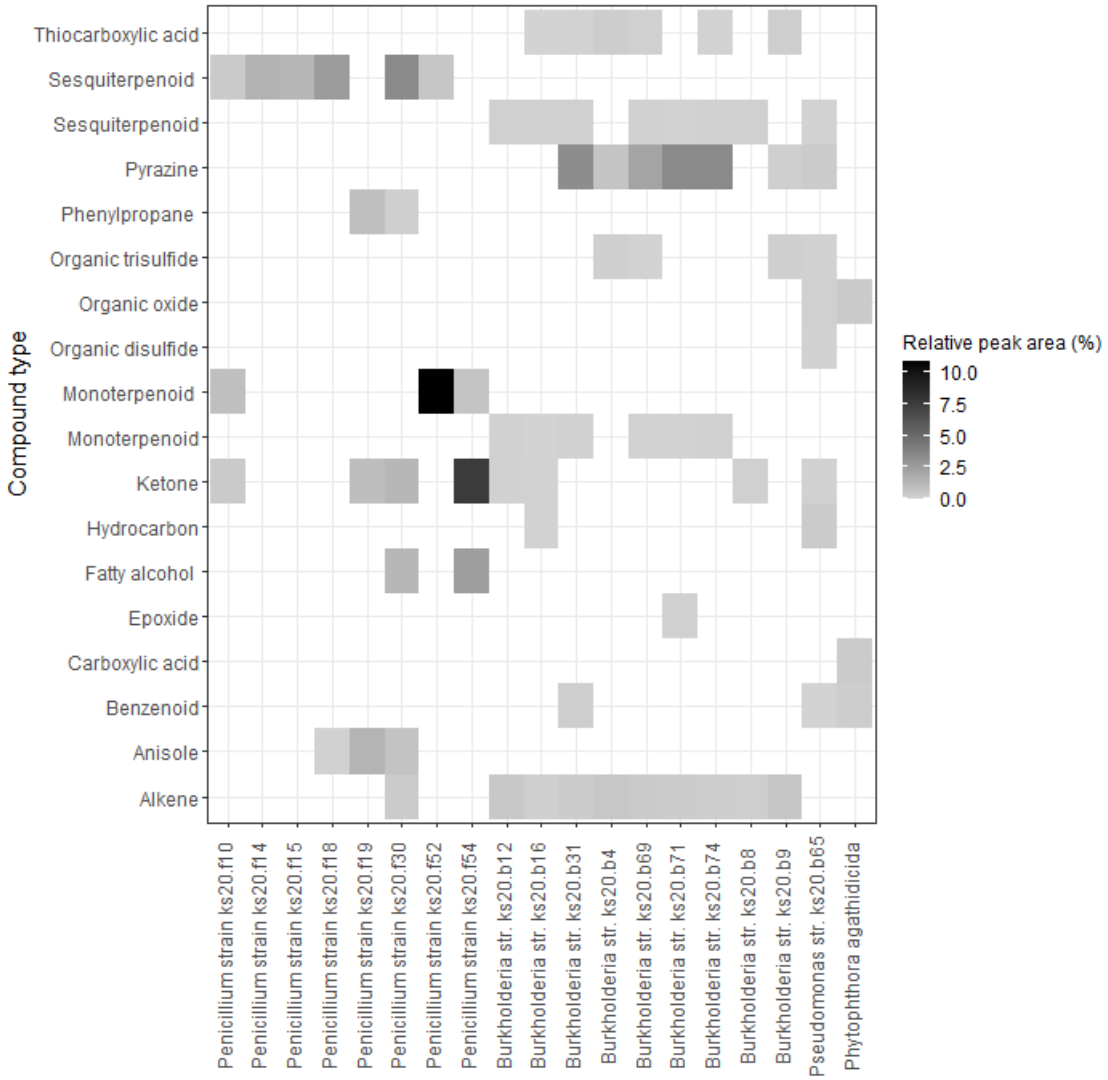

Figure S8 The peak areas (%) of the putatively identified chemical classes detected as being released by fungal isolates, bacterial isolates, and *Phytophthora agathidicida* relative to the total peak area for all compounds using HS SPME-GC-MS analysis. For all samples, siloxane contaminants from the vials constituted most of the VOCs detected in the headspace (not shown).

Table S1 The accepted volatile organic compounds detected in the fungal and bacterial isolates analysed. The molecular weight, molecular formula and literature linear retention index (LRI) of each putatively identified compound is shown, including the associated reference selected as the literature source for the LRI value.

CAS number	Compound Name	Molecular weight	Molecular Formula	Measured LRI	Literature LRI	Reference
15192-80-0	Octa-2,4,6-triene	108	C_8H_{12}	935	925	Mass Spectrometry Data Center (2020)
586-62-9	alpha-Terpinolene	136	$C_{10}H_{16}$	1094	1090	Oliveira et al. (2007)
546-28-1	beta Cedrene	204	$C_{15}H_{24}$	1435	1424	Champagnat et al. (2006)
123-35-3	beta Myrcene	136	$C_{10}H_{16}$	993	981	Kartal et al. (2007)
13877-91-3	beta Ocimene	136	$C_{10}H_{16}$	1050	1050	Juliani and Simon (2002)
17679-93-5	1,3,5-Heptatriene	94	C_7H_{10}	792	781	Pino et al. (2005)
502-99-8	alpha Ocimene	136	$C_{10}H_{16}$	1040	1052	Liu et al. (2006)
99-83-2	alpha Phellandrene	136	$C_{10}H_{16}$	1010	996	Kartal et al. (2007)
40716-66-3	trans-Nerolidol	222	$C_{15}H_{26}$	1570	1564	Mass Spectrometry Data Center (2020)
1461-03-6	beta-Himachalene	204	$C_{15}H_{24}$	1514	1500	Mevy et al. (2006)
3391-86-4	1-Octen-3-ol	128	$C_8H_{16}O$	983	982	Sharififar et al. (2007)
821-95-4	1-Undecene	154	$C_{11}H_{22}$	1091	1087	Beens et al. (1998)
6846-50-0	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	286	$C_{16}H_{30}$	1602	1587	Andriamaharavo (2014)
79-92-5	Camphene	136	$C_{10}H_{16}$	953	933	Kartal et al. (2007)
2460-77-7	2,5-di-tert-Butyl-1,4-benzoquinone	220	$C_{14}H_{20}$	1475	1466	Mass Spectrometry Data Center (2020)
110-43-0	2-Heptanone	114	$C_7H_{14}O$	893	889	Santos et al. (1998)
105-42-0	4-Methyl-2-hexanone	114	$C_7H_{14}O$	853	846	Xu et al. (2003)
821-55-6	2-Nonanone	142	$C_9H_{18}O$	1094	1091	Adams (2000)
2345-28-0	2-Pentadecanone	226	$C_{15}H_{30}$	1702	1698	Schwob et al. (2004)
80-56-8	2-Pinene	136	$C_{10}H_{16}$		939	Hazzit et al. (2006)

0-00-0	2-butan-2-yl-3-methoxy-5-(2-methylpropyl) pyrazine	222	$C_{13}H_{22}N_2O$	1449	1463	Mass Spectrometry Data Center (2020)
106-68-3	3-Octanone	128	$C_8H_{16}O$	992	988	Jean et al. (1993)
0-00-0	4-Chlorobutyric acid, 4-isopropylphenyl ester	240	$C_{13}H_{17}$		1732	Mass Spectrometry Data Center (2020)
100-84-5	3-Methylanisole	122	$C_8H_{10}O$		1029	Jalali-Heravi and Garkani-Nejad (1993)
100-66-3	Methoxybenzene	108	C_7H_8O	923	912	Hoskovec et al. (2005)
18172-67-3	beta-Pinene	136	$C_{10}H16$	981	964	Kartal et al. (2007)
128-37-0	Butylated hydroxytoluene	220	$C_{15}H_{24}$	1525	1514	Adams et al. (2005)
470-40-6	cis-Thujopsene	204	$C_{15}H_{24}$	1445	1426	Skaltsa et al. (2003)
3658-80-8	Dimethyl trisulfide	126	$C_2H_6S_3$	972	971	Pham et al. (2008)
5989-27-5	D-Limonene	136	$C_{10}H_{16}$	1030	1031	Jordan et al. (2002)
124-76-5	Isoborneol	154	$C_{10}H_{18}O$	1167	1160	Baranauskienė et al. (2003)
10482-56-1	alpha-Terpineol	154	$C_{10}H_{18}$	1201	1187	Skaltsa et al. (2003)
20333-39-5	Ethyl methyl disulfide	108	$C_3H_8S_2$	837	833	Misharina and Golovnya (1989)
7289-53-4	1-Methoxyundecane	186	$C_{12}H_{26}O$	1281	1290	Mass Spectrometry Data Center (2020)
2855-19-8	2-Decyloxirane	184	$C_{12}H_{24}$	1313	1307	Yayli et al. (2006)
96-76-4	2,4-Di-tert-butylphenol	206	$C_{14}H_{22}O$	1518	1513	Verekin et al. (1990)
60-12-8	2-Phenylethanol	122	$C_8H_{10}O$	1122	1116	Alissandrakis et al. (2007)
13360-64-0	2-Ethyl-5-methylpyrazine	122	$C_7H_{10}N_2$	1004	1000	Solina et al. (2005)
5925-75-7	S-Methyl propanethioate	104	C_4H_8OS	800	785	Garbuzov et al. (1985)
18431-82-8	beta-Chamigrene	204	$C_{15}H_{24}$	1492	1478	Asuming et al. (2005)
19912-83-5	alpha-Chamigrene	204	$C_{15}H_{24}$	1519	1516	Asuming et al. (2005)
5756-24-1	Dimethyltetrasulfane	158	$C_2H_6S_4$	1222	1200	Misharina and Golovnya (1989)
495-62-5	Bisabolene	204	$C_{15}H_{24}$	1487	1509	Mass Spectrometry Data Center (2020)

Table S2 The volatile organic compounds that were putatively identified to be produced by the microbial isolates tested using HS SPME-GC-MS analysis. Only compounds which had a > 85% match rate and had a linear retention index (LRI) within 2% of their reported literature values are reported.

Microbial isolate	Name	% peak area	Match rate %	Class
Burkholderia ks20_b4	1-undecene (CAS: 821-95-4)	0.56 ± 0.04	98	Alkene
	Dimethyl trisulfide (CAS: 3658-80-8)	0.19 ± 0.01	94	Organic trisulfide
	2-butan-2-yl-3-methoxy-5-(2-methylpropyl) pyrazine (CAS: 870543-98-9)	0.67 ± 0.11	90	Pyrazine
	S-methyl propanethioate (CAS: 5925-75-7)	0.26 ± 0.02	86	Thiocarboxylic acid
	Contaminants detected in control vials	64.46 ± 6.35		
Burkholderia ks20_b8	1-undecene (CAS: 821-95-4)	0.25 ± 0.01	97	Alkene
	2-pentadecanone (CAS: 2345-28-0)	0.14 ± 0.00	96	Ketone
	beta-myrcene (CAS: 123-35-3)	0.12 ± 0.01	95	Monoterpenoid
	Contaminants detected in control vials	45.08 ± 7.55		
Burkholderia ks20_b9	1-undecene (CAS: 821-95-4)	0.60 ± 0.04	97	Alkene
	2-butan-2-yl-3-methoxy-5-(2-methylpropyl) pyrazine (CAS: 870543-98-9)	0.18 ± 0.00	88	Pyrazine
	Dimethyl trisulfide (CAS: 3658-80-8)	0.19 ± 0.03	93	Organic trisulfide
	S-methyl propanethioate (CAS: 5925-75-7)	0.25 ± 0.02	85	Thiocarboxylic acid
	Contaminants detected in control vials	81.12 ± 5.02		
Burkholderia ks20_b12	Beta ocicene (CAS: 123-35-3)	0.13 ± 0.00	96	Monoterpenoid
	beta ocimene (CAS: 3779-61-1)	0.07 ± 0.00	98	Monoterpenoid
	alpha ocimene (CAS: 6874-10-8)	0.03 ± 0.00	97	Monoterpenoid
	1-undecene (CAS: 821-95-4)	0.52 ± 0.01	98	Alkene
	2-pentadecanone (CAS: 2345-28-0)	0.09 ± 0.01	92	Ketone
	Contaminants detected in control vials	31.40 ± 3.03		
Burkholderia ks20_b71	Beta myrcene (CAS: 123-35-3)	0.06 ± 0.01	96	Monoterpenoid
	beta ocimene (CAS: 3779-61-1)	0.04 ± 0.00	98	Monoterpenoid
	1-undecene (CAS: 821-95-4)	0.30 ± 0.04	98	Alkene
	2-butan-2-yl-3-methoxy-5-(2-methylpropyl) pyrazine (CAS: 870543-98-9)	3.48 ± 0.34	91	Pyrazine

Burkholderia ks20_b71	2-decyloxirane (CAS: 2855-19-8)	0.14 ± 0.03	95	Epoxide
	Contaminants detected in control vials	26.90 ± 1.60		
Burkholderia ks20_b74	beta-myrcene (CAS: 123-35-3)	0.10 ± 0.00	96	Monoterpenoid
	beta ocimene (CAS: 3779-61-1)	0.07 ± 0.00	98	Monoterpenoid
	alpha ocimene (CAS: 6874-10-8)	0.03 ± 0.00	98	Monoterpenoid
	1-Undecene (CAS: 821-95-4)	0.28 ± 0.03	98	Alkene
	2-butan-2-yl-3-methoxy-5-(2-methylpropyl) pyrazine (CAS: 870543-98-9)	3.47 ± 0.01	92	Pyrazine
	S-Methyl propanethioate (CAS: 5925-75-7)	0.03 ± 0.01	87	Thiocarboxylic acid
	Contaminants detected in control vials	14.01 ± 2.34		
Burkholderia ks20_b16	beta-myrcene (CAS: 123-35-3)	0.12 ± 0.01	95	Monoterpenoid
	beta ocimene (CAS: 3779-61-1)	0.08 ± 0.00	97	Monoterpenoid
	alpha ocimene (CAS: 6874-10-8)	0.04 ± 0.00	98	Monoterpenoid
	Pentamethylcyclopentadiene (CAS: 4045-44-7)	0.05 ± 0.00	92	Alkene
	1-undecene (CAS: 821-95-4)	0.18 ± 0.01	97	Alkene
	2-pentadecanone (CAS: 2345-28-0)	0.03 ± 0.00	94	Ketone
	D-limonene (CAS: 5989-27-5)	0.02 ± 0.00	96	Monoterpenoid
	S-methyl propanethioate (CAS: 5925-75-7)	0.005 ± 0.00	87	Thiocarboxylic acid
	Contaminants detected in control vials	20.65 ± 17.22		
Burkholderia ks20_b31	beta-myrcene (CAS: 123-35-3)	0.12 ± 0.00	96	Monoterpenoid
	beta ocimene (CAS: 3779-61-1)	0.07 ± 0.01	98	Monoterpenoid
	alpha ocimene (CAS: 6874-10-8)	0.04 ± 0.00	98	Monoterpenoid
	Pentamethylcyclopentadiene (CAS: 4045-44-7)	0.05 ± 0.00	93	Alkene
	1-undecene (CAS: 821-95-4)	0.32 ± 0.02	98	Alkene
	2-butan-2-yl-3-methoxy-5-(2-methylpropyl) pyrazine (CAS: 870543-98-9)	3.33 ± 0.07	92	Pyrazine
	S-methyl propanethioate (CAS: 5925-75-7)	0.05 ± 0.01	86	Thiocarboxylic acid
	Contaminants detected in control vials	11.14 ± 0.55		
Pseudomonas ks20_b65	Octa-2,4,6-triene (CAS: 15192-80-0)	0.37 ± 0.02	97	Terpene
	beta-myrcene (CAS: 123-35-3)	0.05 ± 0.03	92	Monoterpenoid
	1,3,5-heptatriene (CAS: 17679-93-5)	1.72 ± 0.01	97	Alkene

Pseudomonas ks20_b65	2-heptanone (CAS: 110-43-0)	0.25 ± 0.01	97	Ketone
	2-nonanone (CAS: 821-55-6)	0.08 ± 0.00	94	Ketone
	2-methylbutanoic acid (CAS: 116-53-0)	0.22 ± 0.10	95	Fatty acyl
	Dimethyl trisulfide (CAS: 3658-80-8)	1.38 ± 0.03	94	Organic trisulfide
	Ethyl methyl disulfide (CAS: 20333-39-5)	0.08 ± 0.00	91	Organic disulfide
	1-methoxyundecane (CAS: 7289-53-4)	0.14 ± 0.01	90	Organic oxide
	2,4-di-tert-butylphenol (CAS: 96-76-4)	0.03 ± 0.00	92	Benzenoid
	2-ethyl-5-methylpyrazine (CAS: 13360-64-0)	0.37 ± 0.03	95	Pyrazine
	Dimethyltetrasulfane (CAS: 5756-24-1)	0.08 ± 0.03	91	Organic trisulfide
	Contaminants detected in control vials	18.89 ± 4.19		
Burkholderia ks20_b69	2-butan-2-yl-3-methoxy-5-(2-methylpropyl) pyrazine (CAS: 870543-98-9)	2.22 ± 0.03	91	Pyrazine
	beta-myrcene (CAS: 123-35-3)	0.08 ± 0.00	96	Monoterpenoid
	Dimethyl trisulfide (CAS: 3658-80-8)	0.04 ± 0.00	93	Organic trisulfide
	beta ocimene (CAS: 3779-61-1)	0.05 ± 0.01	97	Monoterpenoid
	S-methyl propanethioate (CAS: 5925-75-7)	0.13 ± 0.02	88	Thiocarboxylic acid
	1-undecene (CAS: 821-95-4)	0.43 ± 0.02	98	Alkene
	Contaminants detected in control vials	24.05 ± 2.29		
Penicillium ks20_f15	trans-nerolidol (CAS: 40716-66-3)	1.34 ± 0.26	95	Sesquiterpenoid
	Contaminants detected in control vials	90.78 ± 0.66		
Penicillium ks20_f18	cis-thujopsene (CAS: 470-40-6)	2.60 ± 0.04	95	Sesquiterpenoid
	beta-chamigrene (CAS: 18431-82-8)	2.17 ± 0.12	96	Sesquiterpenoid
	beta-himachalene (CAS: 1461-03-6)	1.27 ± 0.05	91	Sesquiterpenoid
	alpha-chamigrene (CAS: 19912-83-5)	1.19 ± 0.02	96	Sesquiterpenoid
	beta-cedrene (CAS: 546-28-1)	0.10 ± 0.00	92	Sesquiterpenoid
	4-methyl-3-hexanone (CAS: 17042-16-9)	0.94 ± 0.06	97	Ketone
	3-methylanisole (CAS: 100-84-5)	0.10 ± 0.00	97	Benzenoid
	Bisabolene (CAS: 495-62-5)	0.45 ± 0.09	92	Sesquiterpenoid
	Contaminants detected in control vials	21.88 ± 1.88		
Penicillium ks20_f54	3-octanone (CAS: 106-68-3)	7.64 ± 1.30	98	Ketone

Penicillium ks20_f54	1-octen-3-ol (CAS: 3391-86-4)	2.56 ± 0.68	99	Fatty alcohol
	alpha-gurjunene (CAS: 489-40-7)	1.17 ± 0.26	89	Sesquiterpenoid
	beta-myrcene (CAS: 123-35-3)	0.69 ± 0.06	95	Monoterpene
	Contaminants detected in control vials	78.15 ± 14.12		
Penicillium ks20_f10	3-octanone (CAS: 106-68-3)	0.73 ± 0.14	97	Ketone
	beta-ocimene (CAS: 13877-91-3)	0.94 ± 0.18	98	Monoterpene
	4-methyl-2-hexanone (CAS: 105-42-0)	0.43 ± 0.01	93	Ketone
	cis-thujopsene (CAS: 470-40-6)	0.41 ± 0.02	97	Sesquiterpenoid
	Contaminants detected in control vials	90.86 ± 0.77		
Penicillium ks20_f14	beta-himachalene (CAS: 1461-03-6)	2.54 ± 0.07	92	Sesquiterpenoid
	alpha-chamigrene (CAS: 19912-83-5)	1.69 ± 0.27	88	Sesquiterpenoid
	cis-thujopsene (CAS: 470-40-6)	1.56 ± 0.04	95	Sesquiterpenoid
	Bisabolene (CAS: 495-62-5)	1.34 ± 0.02	91	Sesquiterpenoid
	Phenol (CAS: 108-95-2)	0.31 ± 0.07	92	Benzenoid
	beta-cedrene (CAS: 546-28-1)	0.43 ± 0.01	92	Sesquiterpenoid
	Contaminants detected in control vials	52.02 ± 3.09		
Penicillium ks20_f19	Methoxybenzene (CAS: 100-66-3)	1.43 ± 0.13	96	Benzenoid
	3-octanone (CAS: 106-68-3)	0.95 ± 0.29	97	Ketone
	Butylated hydroxytoluene (CAS: 128-37-0)	0.92 ± 0.14	96	Benzenoid
	Contaminants detected in control vials	96.02 ± 0.80		
Penicillium ks20_f30	Methoxybenzene (CAS: 100-66-3)	3.49 ± 0.22	96	Benzenoid
	3-octanone (CAS: 106-68-3)	1.32 ± 0.02	97	Ketone
	1-octen-3-ol (CAS: 3391-86-4)	1.28 ± 0.07	95	Fatty alcohol
	Butylated hydroxytoluene (CAS: 128-37-0)	0.20 ± 0.03	95	Benzenoid
	trans-nerolidol (CAS: 40716-66-3)	0.74 ± 0.05	94	Sesquiterpenoid
	1,3,5-heptatriene (CAS: 17679-93-5)	0.35 ± 0.03	96	Alkene
	Contaminants detected in control vials	85.45 ± 5.37		
Penicillium ks20_f52	D-limonene (CAS: 5989-27-5)	10.85 ± 0.48	96	Monoterpenoid
	Isoborneol (CAS: 124-76-5)	10.81 ± 1.41	97	Monoterpenoid
	Beta-pinene (CAS: 18172-67-3)	2.30 ± 0.07	96	Monoterpenoid

Penicillium ks20_f52	alpha-terpinolene (CAS: 586-62-9)	2.32 ± 0.12	97	Monoterpenoid
	alpha-pinene (CAS: 80-56-8)	1.07 ± 0.02	98	Monoterpenoid
	alpha phellandrene (CAS: 99-83-2)	0.68 ± 0.03	86	Monoterpenoid
	Methoxybenzene (CAS: 100-66-3)	0.60 ± 0.08	94	Benzenoid
	alpha-terpineol (CAS: 10482-56-1)	0.23 ± 0.02	94	Monoterpenoid
	Camphene (CAS: 79-92-5)	6.83 ± 0.24	97	Monoterpenoid
	Contaminants detected in control vials	64.26 ± 1.26		
Phytophthora agathidicida NZFS3770	2,2,4-trimethyl-1,3-pentanediol di-isobutyrate (CAS: 6846-50-0)	0.35 ± 0.03	80	Carboxylic acid
	2,5-di-tert-butyl-1,4-benzoquinone (CAS: 2460-77-7)	0.42 ± 0.06	89	Carbonyl compound
	Methyl salicylate (CAS: 119-36-8)	0.28 ± 0.07	93	Benzenoid
	4-ethylphenol (CAS: 123-07-9)	0.28 ± 0.02	94	Benzenoid
	2-Phenylethanol (CAS: 60-12-8)	0.65 ± 0.05	97	Benzenoid
	Contaminants detected in control vials	88.71 ± 0.93		

Table S3 The mean ± standard error mycelial inhibition values (MIV %) of *P. agathidicida* cultures when grown in dual culture, culture filtrate and split plate bioassays with each of the 9 fungal isolates under study. Significance tested using 2 sample t-tests.

	Dual culture bioassay		**Culture filtrate bioassay**		**Split plate bioassay**	
Fungal isolate ID	**Mycelial inhibition %**	**Significance**	**Mycelial inhibition %**	**Significance**	**Mycelial inhibition %**	**Significance**
Penicillium ks20_f18	58.28 ± 1.55	t-value = 33.80, p-value = 0.000	4.05 ± 2.88	t-value = 1.02, p-value = 0.329	14.41 ± 2.42	t-value= 5.55, p-value = 0.012
Penicillium ks20_f15	48.03 ± 1.71	t-value = 25.74, p-value = 0.000	6.23 ± 3.94	t-value = 1.30, p-value = 0.241	20.26 ± 2.64	t-value= 7.22, p-value = 0.000
Penicillium ks20_f14	54.63 ± 0.80	t-value = 49.48, p-value = 0.000	7.03 ± 2.80	t-value = 1.80, p-value = 0.088	19.53 ± 4.83	t-value= 7.22, p-value = 0.000
Penicillium ks20_f19	52.24 ± 1.47	t-value = 31.57, p-value = 0.000	5.44 ± 2.82	t-value = 1.39, p-value = 0.182	19.09 ± 4.60	t-value= 4.06, p-value = 0.005
Penicillium ks20_f52	55.27 ± 1.26	t-value = 37.60, p-value = 0.000	0.57 ± 2.65	t-value = 0.15, p-value = 0.882	20.33 ± 4.76	t-value= 4.19, p-value = 0.006
Penicillium ks20_f54	51.29 ± 1.40	t-value = 32.25, p-value = 0.000	5.95 ± 2.48	t-value = 1.62, p-value = 0.124	16.91 ± 6.66	t-value= 2.51, p-value = 0.054
Penicillium ks20_f10	50.18 ± 1.74	t-value = 26.50, p-value = 0.000	6.4 ± 2.44	t-value = 1.75, p-value = 0.098	18.68 ± 4.79	t-value= 3.82, p-value = 0.009
Penicillium ks20_f30	57.27 ± 1.20	t-value = 40.46, p-value = 0.000	5.82 ± 2.52	t-value = 1.57, p-value = 0.134	18.94 ± 3.82	t-value= 4.81, p-value = 0.003
Penicillium ks20_f20	52.47 ± 1.49	t-value = 31.42, p-value = 0.000	33.72 ± 4.95	t-value = 5.98, p-value = 0.000	19.90 ± 6.92	t-value= 2.85, p-value = 0.046

Table S4 The mean ± standard error mycelial inhibition values (MIV %) of *P. agathidicida* cultures when grown in dual culture, culture filtrate and split plate bioassays with each of the 11 bacterial isolates under study. Significance tested using 2 sample t-tests.

	Dual culture bioassays		**Culture filtrate bioassays**		**Split plate bioassays**	
Bacterial isolate ID	**Mycelial inhibition %**	**Significance**	**Mycelial inhibition %**	**Significance**	**Mycelial inhibition %**	**Significance**
Burkholderia ks20_b16	57.87 ± 1.26	t-value = 29.61, p-value = 0.000	11.63 ± 3.47	t-value = 2.98, p-value = 0.009	14.77 ± 3.85	t-value= 3.72, p-value = 0.006
Burkholderia ks20_b12	59.91 ± 1.84	t-value = 25.27, p-value = 0.000	10.44 ± 3.96	t-value = 2.40, p-value = 0.031	14.48 ± 3.44	t-value= 4.06, p-value = 0.002
Burkholderia ks20_b4	56.66 ± 2.10	t-value = 22.01, p-value = 0.000	13.58 ± 2.92	t-value = 3.96, p-value = 0.001	19.87 ± 4.49	t-value= 4.33, p-value = 0.003
Burkholderia ks20_b8	55.75 ± 1.81	t-value = 23.74, p-value = 0.000	14.40 ± 2.50	t-value = 4.67, p-value = 0.000	18.75 ± 4.30	t-value= 4.26, p-value = 0.002
Burkholderia ks20_b9	58.13 ± 1.88	t-value = 24.20, p-value = 0.000	16.43 ± 1.71	t-value = 6.62, p-value = 0.000	21.03 ± 3.17	t-value= 6.35, p-value = 0.000
Burkholderia ks20_b69	59.78 ± 1.37	t-value = 29.57, p-value = 0.000	13.69 ± 3.19	t-value = 3.74, p-value = 0.002	16.39 ± 2.73	t-value= 5.69, p-value = 0.000
Pseudomonas ks20_b65	51.88 ± 1.16	t-value = 27.49, p-value = 0.000	13.01 ± 3.31	t-value = 3.46, p-value = 0.003	9.01 ± 6.52	t-value= 1.38, p-value = 0.201
Burkholderia ks20_b31	53.19 ± 1.77	t-value = 22.96, p-value = 0.000	13.4 ± 3.74	t-value = 3.23, p-value = 0.006	15.62 ± 5.31	t-value= 2.89, p-value = 0.018
Burkholderia ks20_b71	60.88 ± 3.22	t-value = 17.15, p-value = 0.000	9.32 ± 2.52	t-value = 3.00, p-value = 0.009	12.25 ± 3.02	t-value= 3.87, p-value = 0.003
Burkholderia ks20_b74	57.31 ± 1.73	t-value = 25.10, p-value = 0.000	10.67 ± 2.88	t-value = 3.14, p-value = 0.005	16.44 ± 2.66	t-value= 5.81, p-value = 0.000
Paraburkholderia ks20_b72	51.52 ± 1.25	t-value = 26.47, p-value = 0.000	6.71 ± 2.70	t-value = 2.07, p-value = 0.059	11.51 ± 3.76	t-value= 2.97, p-value = 0.014

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